GROWTH FACTORS AND CYTOKINES IN HEALTH AND DISEASE

Editors: DEREK LEROITH
CAROLYN BONDY

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IN HEALTH AND DISEASE

A Multi-Volume Treatise

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A Multi-Volume Treatise

CYTOKINES

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PREFACE

Advances in molecular technology in recent years have catalyzed an explosive growth of information about intercellular peptide messengers and their receptors. For example, 10 years ago the only neurotrophin characterized at the molecular level was nerve growth factor (NGF) and the only recognized neurotrophin receptor was the p75 NGF receptor. At present, the number of described neurotrophic peptides approaches 30 and the number of receptors is increasing apace. Just six years ago, the characterized interleukins numbered about three while now there are at least 16. Because many of these new peptide ligands and receptors were identified by "reverse genetic" techniques the understanding of their biological roles lags behind the knowledge of their molecular structures. Over the past few years, however, a new era of functional studies has begun because recombinant proteins have become available for clinical studies. In addition, animal models have been and are being developed using recombinant DNA techniques. Both the clinical studies and studies of transgenic and target deleted mice will allow for further physiologic elucidation of the biological roles of these messenger peptides and their receptors.

This series of growth factors and cytokines is divided into three main sections: Growth Factors (Volumes 1A and 1B), Cytokines (Volumes 2A and 2B), and Systems (Volumes 3A and 3B). Although Volumes 1 and

2 are separate the distinction between "growth factors" and "cytokines" is probably more historical or pragmatic than indicative of differences in function. The term "growth factors" refers to a wide variety of locally or systemically produced proteins with pleiotropic actions on tissue growth and differentiation. The term "cytokines" describes a group of proteins identified primarily within the immune and hematopoietic systems, although it is likely that such a narrow view of cytokines will not survive for long. For example, it appears that some interleukins and interleukin receptors are expressed by neuroepithelial cells *in vivo* suggesting that these interleukins may have intrinsic roles within the nervous system. Furthermore, tumor necrosis factor (TNF) has been identified as a potential adipose tissue regulatory factor which is both produced and acts locally. The third volume titled *Systems* deals more directly with the role of these factors in both normal physiology and the disease processes resulting from the deficiency or excess of growth factors/cytokines and their receptors.

This second volume deals with cytokines and their receptors. The field is advancing at a rapid pace and we have attempted to cover as many of the cytokines as possible. Interleukins 11-15, in fact, will be discussed under "The Immune System" in the third volume. While we have attempted to be as comprehensive and inclusive as possible, there will always be some regrettable omissions. At the publishing date we recognize that a few growth factors and cytokines have not been included in this review. These new discoveries will for certain be reviewed in similar pages in the future.

Derek LeRoith
Carolyn Bondy
Editors

THE TYPE I INTERFERON RECEPTOR COMPLEX

Sidney Pestka

Growth Factors and Cytokines in Health and Disease
Volume 2B, pages 403-431.

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I. INTRODUCTION

The interferons (IFNs) consist of a group of cytokines that can be divided into two groups designated Type I and Type II interferons. IFN-γ is the only Type II interferon whereas the Type I interferons consist of four major classes: IFN-α, IFN-β, IFN-ω, and IFN-τ. In the case of the human interferons, there is only one functional representative of the IFN-β and IFN-ω class, but a family of multiple IFN-α species (about 12 separate expressed proteins). It is not clear how many human IFN-τ species exist, but there may be one (Whaley et al., 1994). The Type I interferon family (IFN-α, IFN-β, IFN-ω, and IFN-τ) are involved in a variety of physiological responses. They exhibit antiviral and antiproliferative activity, stimulation of cytotoxic activity of lymphocytes, natural killer cells and macrophages, modulation of cellular differentiation and stimulation of class I MHC antigens and other surface markers, and activation of the JAK-STAT signal transduction pathway (Lengyel, 1982; Pestka et al., 1987; Langer and Pestka, 1988; Sen and Lengyel, 1992; Darnell et al., 1995; Uzé et al., 1995). Similar to most cytokines and growth factors the actions of Type I, IFNs are mediated by interaction with specific cell surface receptors (Friedman, 1967; Aguet, 1980). Competition binding studies demonstrated that Type I IFNs share the same receptor complex, whereas Type II IFN (IFN-γ) binds to a distinct receptor (Branca and Baglioni, 1981; Pestka et al., 1987; Flores et al., 1991; Li and Roberts, 1994; Izotova, Mariano, Roberts, Li, and Pestka, unpublished observations). Components of both these classes of receptors were cloned. A functional Type II receptor requires two transmembrane chains (Aguet et al., 1988; Kumar et al., 1989; Soh et al., 1993, 1994a; Hemmi et al., 1994). Two chains of the Type I receptor appear to have been cloned (Uzé et al., 1990; Novick et al., 1994), but identification of all the components of a fully functional Type I receptor and determination of the role of each component are yet to be achieved. As described in the following sections, we have been able to obtain a functional Type I receptor (Soh et al., 1994c), however, isolation of all the multiple components of this receptor is still to be accomplished. This review will focus chiefly on the Type I interferon receptor.

Table 1. Designations of Human Interferon Receptor Components

Type I Interferon (IFN-α/β/ω/τ) Receptor	
Chain or Clone	*References*
Hu-IFN-αR1 (IFNAR)	Uzé et al. (1990)
Hu-IFN-αR2	Novick et al. (1994)
αYAC (F136C5)	Soh et al. (1994c)
Type I Interferon (IFN-γ) Receptor	
Chain	*References*
Hu-IFN-γ R1 (Hu-IFN-γ Rα; ligand binding chain of receptor)	Aguet et al. (1988), Kumar et al. (1989), Hemmi et al. (1989), Gray et al. (1989), Munro and Maniatis, (1989), Cofano et al. (1990)
Hu-IFN-γ R2 (Accessory Factor-1; AF-1; Hu-IFN-γ R β; second chain of receptor)	Soh et al. (1994a), Hemmi et al. (1994)

II. RECEPTOR NOMENCLATURE

The designation of the interferon receptor components for the purposes of this discussion are given in Table 1. Both Type I and Type II interferon receptor components have been listed for comparison. These receptor complexes consists of two or more components. It also appears that the individual components may contribute to one extent or another to ligand binding so that designations such as an α subunit for the ligand binding component and the β subunit for the signal transduction subunit are not warranted. In addition, it is not yet clear what the functions of each subunit are and, indeed, exactly how many subunits there are. For this reason, the subunits are named in the order they have been cloned and discovered as distinct entities. Alternative designations for the subunits that have been used are also given in parentheses (Table 1).

III. CHROMOSOMAL LOCALIZATION, ANTIBODIES, AND CDNA CLONES

Somatic cell genetic studies with human x rodent hybrid cells containing various combinations of human chromosomes have provided evidence that the presence of human Chromosome 21 confers sensitivity of the rodent cells to human Type I interferons (Tan et al., 1973; Slate et al., 1978; Epstein et al., 1982; Raziuddin et al., 1984). It was also demon-

strated that antibodies to human Chromosome 21-encoded cell surface components were able to block the action or binding of Hu-IFN-α to cells (Revel et al., 1976; Shulman et al., 1984). Later, Langer et al. (1990) demonstrated that 3x1S irradiation-reduced hamster x human somatic hybrid cells containing about 3 mb of Chromosome 21q around 21q22.1 (Jung, 1991; Soh et al., 1994c) were able to bind [^{32}P]Hu-IFN-αA and generate a complex of about 150 kDa when cross-linked to the cell surface. During this time, the gene for the Hu-IFN-αR1 receptor chain was mapped to the 3x1S region (21q22.1; Lutfalla et al., 1990). The paradoxical observation that CHO 3x1S cells could bind Hu-IFN-αA whereas the expression of the cloned receptor cDNA (Hu-IFN-αR1) in mouse cells did not confer binding to Hu-IFN-αA suggested that the 3x1S region of human Chromosome 21 contains other subunits of the receptor complex. This assumption was supported by the identification of two separate components following immunoprecipitation of [^{125}I]Hu-IFN-αA:receptor complexes from 3x1S cell extracts with anti-IFN-α receptor antibody (Colamonici and Domanski, 1993; Colamonici et al., 1990, 1992). These two subunits differ from the cloned Hu-IFN-αR1. The monoclonal antibodies (MAbs) against one subunit (110 kDa) and the recombinant Hu-IFN-αR1 receptor blocked the biological activity of Type I interferons while MAbs against the second subunit did not (Colamonici and Domanski, 1993; Benoit et al., 1993), suggesting that the Type I IFN receptor consists of at least two different subunits. Antibodies to Hu-IFN-αR1 blocked the activity of various Type I interferons (Uzé et al., 1991). Thus, it is likely that the Hu-IFN-αR1 molecule is at least one component of the Type I IFN receptor.

Mouse cells with functional Hu-IFN Type I IFN receptors were first isolated by Jung and Pestka (1986) and by Revel et al. (1991). The mouse cells transfected with total human DNA exhibited the properties of a Type I interferon receptor (also designated Hu-IFN-αR, Hu-IFN-βR, or Hu-IFN-α/βR) in that they responded to both Type I interferons tested—Hu-IFN-α and Hu-IFN-β. Although primary transformants were obtained by these groups, no molecular clone was obtained that provided Type I interferon receptor activity. Employing the procedures of Jung and Pestka (1986), Uzé et al. (1990) were able to obtain a molecular clone that they designated the Type I IFN receptor by switching the selection procedure from Hu-IFN-αA and Hu-IFN-β to Hu-IFN-αB2 (a more species-specific Hu-IFN-α). However, as shown by them and other groups, this cDNA clone, when expressed in mouse or hamster cells, did not bind all Type I interferons other than Hu-IFN-αB2 and

yielded very little response even to Hu-IFN-aB2. None of the other Hu-IFN-a species tested seemed to activate the mouse cells expressing this cDNA clone. During this period, however, a number of groups obtained antibodies suggesting that the Type I receptor consisted of multiple components, a hypothesis consistent with the inability of Jung and Pestka (1986) to obtain stable secondary transformants. A comparison of hamster cells with the 3x1S region of human Chromosome 21 and cells transfected with the Hu-IFN-αR1 cDNA (the clone described by Uzé et al., 1990) showed that other components were involved in the Type I interferon receptor. Hamster cells with the 3x1S region of human DNA responded to Hu-IFN-αA and Hu-IFN-β whereas mouse or hamster cells with the Hu-IFN-αR1 cDNA did not respond to low concentrations of these interferons (Langer et al., 1990; Soh et al., 1994c).

As noted above, cloning of the first human Type I IFN receptor chain (IFN-αR1) was reported on the basis of rendering mouse cells sensitive to Hu-IFN-αB2 (Uzé et al., 1990). However, when mouse cells were transfected with the Hu-IFN-αRF cDNA, they did not exhibit binding and antiviral protection with Type I IFN subtypes other than Hu-IFN-αB2. Similarly, human cells transfected with the homologous cloned Mu-IFN-αR1 receptor cDNA showed antiviral protection activity with only Mu-IFN-α11. However, the expression of this Mu-IFN-αR1 cDNA in murine L1210 R101 cells resistant to Type I IFNs and lacking mRNA for this Mu-IFN-αR1 receptor component showed antiviral protection in response to all Type I Mu-IFNs tested (Uzé et al., 1992). In contrast, it was reported that when Chinese hamster ovary (CHO-K1) cells were transfected with the cloned Hu-IFN-αR1 cDNA, no induction of 2'-5'A synthetase activity was observed after treating cells with Hu-IFN-αA and Hu-IFN-αB2 (Revel et al., 1991).

In affinity cross-linking experiments, [^{125}I]Hu-IFN-α:receptor complexes with M_r of 80,000 (Hannigan et al., 1986), 210,000 (Colamonici et al., 1992), 260,000 (Vanden Broeke et al., 1988), or 300,000 (Raziuddin and Gupta, 1985) were observed in addition to the major complex which migrates as a broad band with a M_r of 140,000-150,000. These observations strongly suggested that other subunits, components, or accessory proteins are involved in ligand binding and in signal transduction in response to Type I IFNs (reviewed by Colamonici and Pfeffer, 1991; Mariano et al., 1992) as described for the IFN-γ receptor (Rashidbaigi et al., 1986; Jung et al., 1987; Cook et al., 1992, 1994; Soh et al., 1993, 1994a).

IV. FUNCTIONAL YAC SELECTION AND SCREENING (FYSS)

During a period of several years, Jung and coworkers (Jung et al., 1987, 1988, 1990; Jung, 1991) attempted many avenues to obtain the gene and/or cDNA clone for Hu-IFN-γ R2 in studies that would serve as a foundation for isolation of a genomic clone encoding the functional Type I IFN receptor complex. The use of cDNA expression libraries, cosmid genomic clones, and total human DNA used for transfection of cells proved inadequate to obtain a molecular clone for Hu-IFN-γR2. Thus, we turned to the use of yeast artificial chromosomes (YACs) since they contain large insertions of human DNA. Yeast artificial chromosome (YAC) cloning techniques allow the cloning of DNA fragments from 100 to 2000 kb (Burke et al., 1987; Chumakov et al., 1992a). The large insertion size can facilitate not only physical mapping of chromosomes due to the smaller number of clones to align (Chumakov et al., 1992b), but can also make it possible to express genes which are larger than conventional cloning procedures permit and regions of chromosomes containing multiple genes. We already demonstrated that YAC clones can be used to express genes by phenotypic mapping in conjunction with conventional yeast genetic techniques such as homologous recombination and spheroplast fusion (Soh et al., 1993; Cook et al., 1994) in the absence of any specific DNA mapping or sequence information. We already knew from the work of Langer et al. (1990) and Jung (1991) that the region of human Chromosome 21 required for Hu-IFN-γR2 activity encompassed no more than 3 mb. Thus, we were encouraged that the use of YACs might delineate the required gene. In order to use YACs for fusion to mammalian cells and selection it was necessary to devise and construct plasmids that would insert mammalian markers into the YACs so that the appropriate cells could be selected (Soh et al., 1994b; Emanuel et al., 1995). In addition, we designed vectors that would fragment the YAC clones into more manageable smaller ones that could be analyzed more effectively (Cook et al., 1993, 1994; Emanuel et al., 1995). The time spent in constructing these vectors proved worthwhile. By examining a number of YACs containing human DNA inserts from this region we were able to find one that contained the gene for Hu-IFN-γR2 chain, the second chain required for function of the human interferon gamma receptor (Soh et al., 1993). This YAC clone then permitted us to obtain cDNA clones for Hu-IFN-γR2 (Soh et al., 1994a). Having successfully used this approach to obtain a functional Hu-IFN-γ receptor, we then

applied this YAC technology to examine the Type I interferon system as described below.

V. A FUNCTIONAL HUMAN INTERFERON TYPE I RECEPTOR

With the knowledge that the 3x1S region of human Chromosome 21 contained the genes for the Type I interferon receptor, we screened three YAC clones from this region. Two YACs were initially screened by a primer pair derived from the 524-5P probe (21S58 locus) near the cloned Hu-IFN-αR1 receptor gene (Lutfalla et al., 1992). Tassone et al. (1990) reported that the two loci (21S58 and IFNAR) were located within 170 kb of each other. It was also reported that the CRFB4 gene identified by the 524-5P probe was located at less than 35 kb from Hu-IFN-αR1 gene (Lutfalla et al., 1993). The αYAC containing the gene for the cloned Hu-IFN-αR1 receptor cDNA was selected for expression into hamster cells based on the assumption that all of the genes involved in forming a functional Type I IFN receptor might be encompassed on this YAC. The objective was to obtain a YAC clone that would support Type I human interferon activity when transferred into hamster cells. We found one YAC clone that fit our criteria. It contained the genes for the cloned Hu-IFN-αR1 receptor subunit and other genes in the 3x1S region of Chromosome 21. In addition, the introduction of this YAC clone, designated αYAC, into hamster cells rendered the cells much more sensitive to Hu-IFN-αA and Hu-IFN-αB2, and somewhat more sensitive to Hu-IFN-ω and Hu-IFN-β (Soh et al., 1994c). Thus, we hypothesized that this YAC clone must contain multiple genes required to reconstitute a fully-functional Type I IFN receptor (Table 1).

VI. ANTIVIRAL PROTECTION OF HAMSTER CELLS CONTAINING THE HU-IFN-αbR1 CDNA OR THE αbYAC

The hamster cells fused with the αYAC containing the Type I interferon receptor complex showed increased sensitivity to Hu-IFN-αA and Hu-IFN-αB2 (Figures 1 and 2) in antiviral protection against EMCV and VSV when compared with the parental hamster 16-9 cells or hamster cells transfected with the Hu-IFN-αR1 receptor cDNA (Soh et al., 1994c). The hamster cells containing the αYAC exhibited large increases

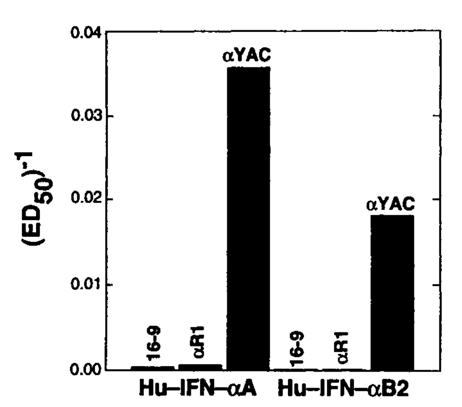


Figure 1. The data in the figure represent the reciprocal of the IFN titer (units/ml) for 50% protection of cells (ED_{50}) against EMCV. The 16-9 cell line is a human x hamster hybrid containing the long arm of human Chromosome 6 and a transfected HLA-B7 gene (Soh et al., 1993). The data for both Hu-IFN-αA and Hu-IFN-αB2 are shown here. Similar data were obtained with parental CHO-K1 hamster cells. Data taken from Soh et al. (1994c).

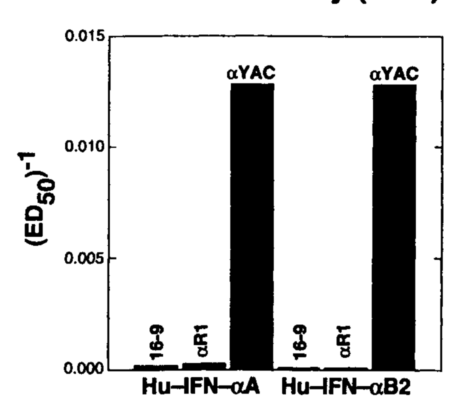


Figure 2. The data in the figure represent the reciprocal of the IFN titer (units/ml) for 50% protection of cells (ED_{50}) against VSV. The experiments were performed as described in the legend to Figure 1 except that VSV was used instead of EMCV. The data for both Hu-IFN-αA and Hu-IFN-αB2 are shown. Data taken from Soh et al. (1994c).

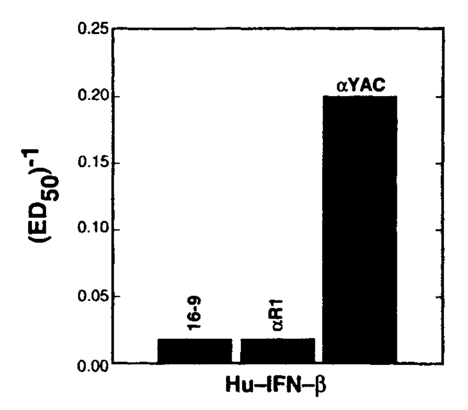


Figure 3. The data in the figure represent the reciprocal of the IFN titer (units/ml) for 50% protection of cells (ED_{50}) against EMCV. The experiments were performed as described in the legend to Figure 1 except that Hu-IFN-β was used instead of the alpha interferons. The value for the $(ED_{50})^{-1}$ for the cells containing the aYAC is >0.20 shown in the figure as maximal protection was obtained at five units/ml of Hu-IFN-β, the lowest Hu-IFN-β concentration tested: therefore, the endpoint (ED_{50}) was <5 units/ml and thus the $(ED_{50})^{-1}$ is >0.20. Accordingly, the value for the cells containing the αYAC as shown is a minimal value. Data taken from Soh et al. (1994c).

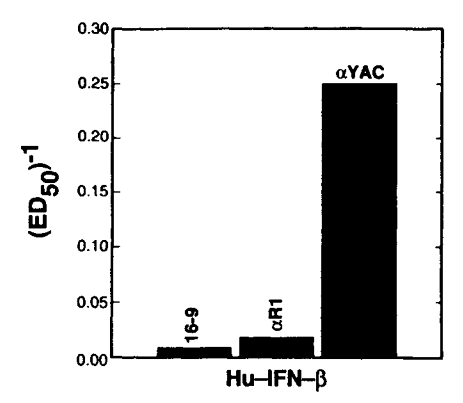


Figure 4. The data in the figure represent the reciprocal of the IFN titer (units/ml) for 50% protection of cells (ED_{50}) against VSV. The experiments were performed as described in the legend to Figure 2 except that Hu-IFN-β was used instead of the alpha interferons. Data taken from Soh et al. (1994c).

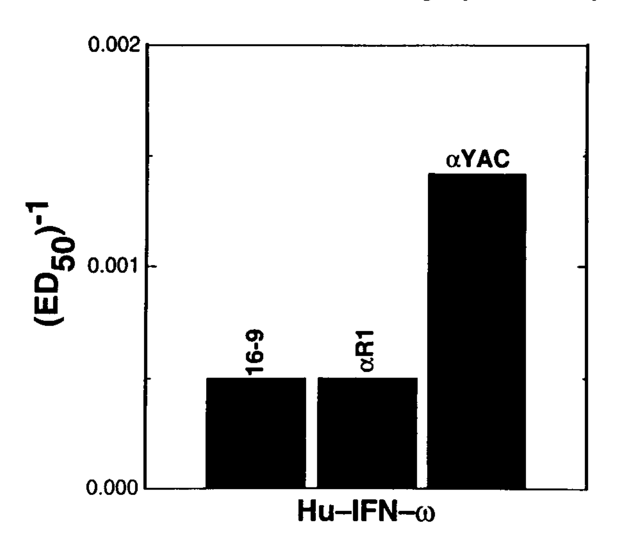


Figure 5. The data in the figure represent the reciprocal of the IFN titer (units/ml) for 50% protection of cells (ED_{50}) against EMCV. The experiments were performed as described in the legend to Figure 1 except Hu-IFN-ω was used instead of the alpha interferons. The value for the $(ED_{50})^{-1}$ for the 16-9 cells and 16-9/αRc5 cells is <0.0005 shown in the figure as the maximal Hu-IFN-ω concentration tested was 2000 units/ml of Hu-IFN-ω. Therefore, the endpoint (ED_{50}) was >2000 units/ml and thus the $(ED_{50})^{-1}$ is <0.0005. Accordingly, the values for these two cell lines are maximum values. Data taken from Soh et al. (1994c).

in sensitivity to Hu-IFN-αA and Hu-IFN-αB2. Furthermore, there was substantial increase in sensitivity to Hu-IFN-β (Figures 3 and 4) and to Hu-IFN-ω (Figures 5 and 6). When the sensitivity of the cells to the interferons was evaluated by assessing the reciprocal of the ED_{50} for protection, in all cases hamster cells with the αYAC showed substantial increase in sensitivity to all these interferons ranging from about threefold to greater than 100-fold (Figures 1-6). There was little or no increased sensitivity of the comparable cells containing the cloned Hu-IFN-αR1 cDNA.

VII. INDUCTION OF CLASS I MHC SURFACE ANTIGENS

Subclones isolated from teams formed hamster 16-9 cells, which express the human HLA-B7 antigen, fused to the αYAC or transfected with the

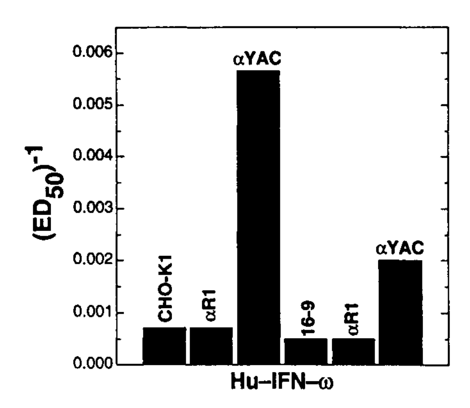


Figure 6. The data in the figure represent the reciprocal of the IFN titer (units/ml) for 50% protection of cells (ED_{50}) against VSV. The experiments were performed as described in the legend to Figure 2 except that Hu-IFN-ω was used instead of the alpha interferons. In addition, data with both CHO-K1 and 16-9 cells are shown for illustration. The first three values of the histogram (left) represent parental CHO-K1 cells, CHO-K1 cells transfected with the Hu-IFN-αR1 cDNA (αR1) and CHO-K1 cells containing the αYAC. The second three values of the histogram (right) represent parental 16-9 cells, 16-9 cells transfected with the Hu-IFN-αR1 cDNA (αR1) and 16-9 cells containing the αYAC. The value for the $(ED_{50})^{-1}$ for the 16-9 cells and 16-9/αRc5 cells is <0.0005 shown in the figure as the maximal Hu-IFN-ω concentration tested was 2,000 units/ml of Hu-IFN-ω. Therefore, the endpoint (ED_{50}) was >2000 units/ml and thus the $(ED_{50})^{-1}$ is <0.0005. Accordingly, the values for these two cell lines are maximum values. The values for the CHO-K1, αR1 and αYAC in CHO-K1 cells (first three values shown in the figure) are correct as the endpoints of the assays fell within the range tested. Data taken from Soh et al. (1994c).

Hu-IFN-αR1 cDNA were tested for class I MHC antigen induction. Table 2 summarizes the HLA-B7 induction as a function of IFN concentration for all of the Type I IFNs tested. At 1, 3, 10, 30, and 100 units/ml, Hu-IFN-αA, Hu-IFN-αB2, Hu-IFN-β, and Hu-IFN-ω had little or no effect on the parental hamster 16-9 cells or hamster 16-9 cells transfected with the Hu-IFN-αR1 cDNA (16-9/αRc5). However, hamster cells fused to the αYAC (16-9/αRy9-2) showed enhanced levels of HLA-B7 antigens in response to Hu-IFN-αA, Hu-IFN-αB2, Hu-IFN-β, or Hu-IFN-ω (Table 2). Treatment of hamster cells fused with the αYAC (16-9/αRy9-2) with as little as 1 unit/ml of Hu-IFN-αA, Hu-IFN-αB2, Hu-IFN-ω, or Hu-IFN-β gave a significant HLA-B7 induction whereas parental hamster 16-9 cells or 16-9 cells transfected with the Hu-IFN-αR1 cDNA (16-9/αRc5) did not show any significant induction at this level (Table 2).

Table 2. Induction of HLA B7 Antigen by Human Type I IFNs

IFN		*Cell Line*		
Units/ml	*Type*	*Hamster 16-9 (Parental)*	*16-9/αRc5 (Hu-IFN-αR1 cDNA)*	*16-9/αRy9-2 (α YAC)*
0	None	1.0	1.0	1.0
1	αA	1.0	0.9	2.0
3	αA	0.8	0.7	1.4
10	αA	0.8	1.0	2.2
30	αA	0.9	0.8	2.0
100	αA	1.0	0.9	2.6
1	αB2	0.9	0.8	1.4
3	αB2	0.8	0.8	2.1
10	αB2	0.8	0.8	1.7
30	αB2	0.6	1.0	2.5
100	αB2	0.9	0.6	2.8
1	ω	1.2	1.0	1.7
3	ω	1.0	1.2	3.0
10	ω	0.9	1.1	3.0
30	ω	0.7	1.3	3.6
100	ω	0.8	1.4	4.3
1	β	0.9	1.1	1.8
3	β	1.0	0.8	1.9
10	β	1.0	1.0	2.0
30	β	1.0	1.2	2.4
100	β	1.6	1.5	2.0

Note: The values in the table represent the average fluorescence displayed by the treated cells divided by the average fluorescence displayed by untreated cells, which were defined as having a relative average fluorescence of 1.0. The assays were all performed at one time except for the results with Hu-IFN-ω that were performed in a separate experiment. Data taken from Soh et al. (1994c).

VIII. CELLS CONTAINING THE αYAC CAN BIND $[^{32}P]$HU-IFN-αA AND $[^{32}P]$HU-IFN-αB2

Antiviral protection and class I MHC antigen induction exhibited by Type I interferons in hamster 16-9 cells containing the αYAC suggested that these cells could bind the Type I interferons. To test this, cells were incubated with $[^{32}P]$Hu-IFN-αA and $[^{32}P]$Hu-IFN-αB2 at room temperature to measure binding of these ligands. As shown in Figures 7 and 8, the hamster cells with the αYAC bound $[^{32}P]$Hu-IFN-αA-P1 and $[^{32}P]$Hu-IFN-αB2-P quite well. There was a small level of binding of $[^{32}P]$Hu-IFN-αB2-P to cells containing the Hu-IFN-αR1 cDNA, but no

binding of [^{32}P]Hu-IFN-αA-P1 was seen. However, no significant binding of [^{32}P]Hu-IFN-αA-P1 and [^{32}P]Hu-IFN-αB2-P to either parental hamster 16-9 or 16-9/YAC-JS2 control cells was observed. The 16-9/YAC-JS2 cells are 16-9 cells fused with the irrelevant GART YAC D142H8.neo.18 (Soh et al., 1993). As a positive control for the binding assay human Daudi cells were used. The level of IFN binding to the hamster cells with the αYAC was about one-fourth to one-tenth of that of Daudi cells. The hamster cells containing the Hu-IFN-αR1 cDNA did not bind Hu-IFN-αA, but did bind Hu-IFN-αB2 slightly (Figures 7 and 8). Furthermore, competition binding studies with [^{32}P]Hu-IFN-αA-P1 as the labeled ligand showed that Hu-IFN-αA, Hu-IFN-αB2, Hu-IFN-β, and Hu-IFN-ω all compete effectively for [^{32}P]Hu-IFN-αA-P1 binding to hamster cells with the αYAC as well as to human Daudi lymphoblastoid cells (Soh et al., 1994c). The specific binding of Hu-IFN-αA and Hu-IFN-αB2 to cells containing the αYAC and the competition by all Type I interferon classes together with the activity data (Figures 1-6 and Table 2) indicate that the human DNA insertion in this YAC contains the genes encoding the subunits comprising a functional Type I receptor. Since the Hu-IFN-αR1 gene is included in this DNA insert, one or more additional genes complementing the cloned Hu-IFN-αR1 receptor en-

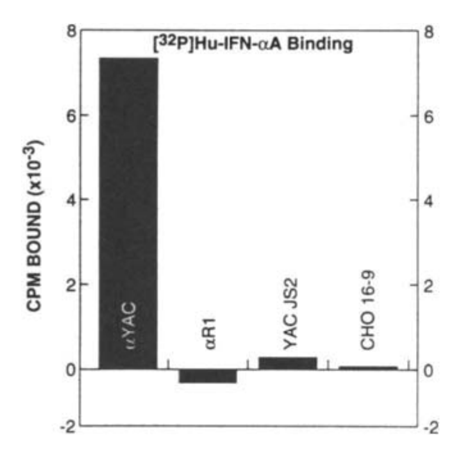


Figure 7. The binding of [^{32}P]Hu-IFN-αA-P1 to various cells is shown. The CHO 16-9 cells are the parental cells for all the other transformants. The cells are as follows: CHO 16-9, the parental cells; YAC JS2, CHO 16-9 cells fused to the irrelevant control YAC JS2 (Soh et al., 1993); αR1, CHO 16-9 cells transfected with the Hu-IFN-αR1 cDNA clone (Uzé et al., 1990); αYAC, CHO 16-9 cells fused to the YAC encompassing the Type I interferon receptor complex. Data taken from Soh et al. (1994c).

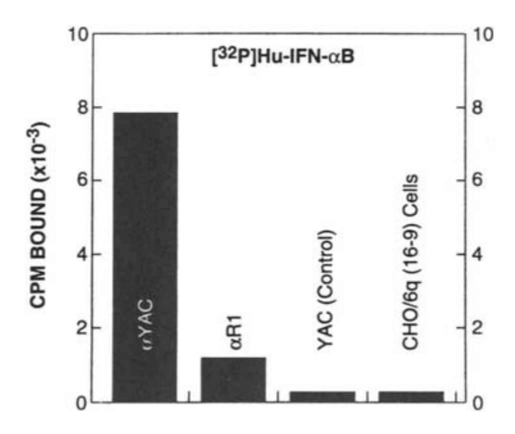


Figure 8. The binding of [^{32}P]Hu-IFN-αB2 to the various cells is shown. The CHO 16-9 cells are the parental cells for all the other transformants. The cells are as follows: CHO 16-9, the parental cells; YAC JS2, CHO 16-9 cells fused to the irrelevant control YAC JS2 (Soh et al., 1993); αR1, CHO 16-9 cells transfected with the Hu-IFN-αR1 cDNA clone (Uzé et al., 1990); αYAC, CHO 16-9 cells fused to the YAC encompassing the Type I interferon receptor complex. Data taken from Soh et al. (1994c).

coded on this YAC enable the cells to bind these interferons effectively (Soh et al., 1994c).

Scatchard analyses of the Hu-IFN-αA and Hu-IFN-αB2 binding data are summarized in Table 3. The data show that the number of binding sites per cell was four to 10-fold higher in Daudi cells than in hamster cells with the αYAC. The dissociation constant (K_d) of the binding of the IFNs to the hamster cells with the αYAC (CHO-K1/αRy9-4) was three to fivefold higher than that to Daudi cells.

IX. PROOF THAT THE IFN-αR1 COMPONENT IS PART OF THE TYPE I RECEPTOR COMPLEX

A large number of experiments were consistent with the concept that the IFN-αR1 chain was one component of the Type I receptor complex. IFN-αR1, as part of the Type I interferon receptor complex, appears to play a role in both ligand binding and intracellular signaling. Previous studies suggested a role of the IFN-αR1 chain in ligand binding and signal transduction: (1) Antibodies to Hu-IFN-αR1 can block the binding of IFN-αs, IFN-β, or IFN-ω to human cells, and act as an antagonist

Table 3. Summary of Comparison of Hu-IFN-αA and [^{32}P]Hu-IFN-αB2 Binding to CHO-K1/αRy9-4 and Daudi Cells

	Hu-IFN-αA		*Hu-IFN-αB2*	
Cell Line	*K_d (M)*	*Binding Sites/Cell*	*K_d (M)*	*Binding Sites/Cell*
CHO-K1/αRy9-4	1.1×10^{-9}	2.3×10^{3}	36×10^{-10}	1.7×10^{3}
Daudi	2.1×10^{-10}	2.3×10^{4}	1.1×10^{-10}	6.2×10^{3}

Notes: Binding data were analyzed by the method of Scatchard (1949) with the use of the Cricket Graph 132 program. CHO-K1/αRy9-4 cells represent a clone of parental hamster CHO-K1 cells containing the αYAC. Data taken from Soh et al. (1994c).

for Type I IFN action (Uzé et al., 1991; Benoit et al., 1993). (2) Murine cells (L1210R) which do not specifically bind Mu-IFN-α and are insensitive to the actions of murine IFN-α or IFN-β regain antiviral and antiproliferative sensitivity to Mu-IFN-a and Mu-IFN-β after transfection with vectors expressing Mu-IFN-αR1 (Uzé et al., 1992). (3) Hu-IFN-αR1 expressed in *Xenopus* oocytes to minimize the possibility of endogenous receptor components can bind and be covalently crosslinked to Hu-IFN-αA and Hu-IFN-αB (Lim et al., 1994). (4) The Hu-IFN-αR1 chain can be covalently crosslinked to various IFN-αs and IFN-β and immunoprecipitated with several monoclonal antibodies (Colamonici et al., 1990; Abramovich et al., 1994). (5) The bovine IFN-αR1 has intrinsic high-affinity binding for several human IFN-αs when expressed on simian COS cells or when expressed in *Xenopus* oocytes (Lim and Langer, 1993; Lim et al., 1994). Some evidence, however, suggests that IFN-αR1 on human cells may not be absolutely required for cellular binding, although the binding affinities have not been determined in most cases. Thus, hamster cells transfected with a Chromosome 21-derived YAC with a deletion in the Hu-IFNAR1 gene retain their binding of human Type I IFNs (Cleary et al., 1994; and see following). Also, IFN-α-resistant K562 cells can bind Type I IFNs, but are restored to IFN-α sensitivity by the introduction of Hu-IFN-αR1, without any apparent change in receptor number or affinity, suggesting more of a role for IFN-αR1 in cellular signaling (Colamonici et al., 1994a).

Evidence for the role of IFN-αR1 in signal transduction includes the enhanced sensitivity to IFNs seen in some transfection studies, sometimes in the absence of enhanced IFN binding (Colamonici et al., 1994a; Constantinescu et al., 1994). The basis for the role of IFN-αR1 in signaling may be its association with the intracellular tyrosine kinase Tyk2 required for IFN-α responses (Velazquez et al., 1992; Barbieri et

al., 1994) as demonstrated by co-immunoprecipitation experiments and *in vitro* binding of purified Tyk2 to a fusion protein including the cytoplasmic domain of IFN-αR1 (Colamonici et al., 1994b).

Direct proof of the requirement for the IFN-αR1 chain for Type I interferon receptor function was demonstrated by experiments disrupting the IFN-αR1 gene. Homozygous deletions of IFNAR1 in mice (IFN-$\alpha R^{0/0}$) were reported by Müller et al. (1993), and shown to cause enhanced susceptibility to several viruses. Primary embryo fibroblasts from such mice lack Type I IFN-stimulated resistance to viruses and inducibility of 2',5'A synthetase. Using homologous recombination targeted to the αYAC, Cleary et al. (1994) produced a deletion within the human IFN-α receptor (Hu-IFN-αR1) gene which eliminated exon II of the gene. The resultant ΔαYAC was transferred to CHO cells. This deletion effectively eliminated the ability of Type I interferons to induce MHC Class I antigens and exhibit antiviral activity which are properties of the fully functional parental YAC clone (Soh et al., 1994c). By subsequent transfection of the cDNA for Hu-IFN-αR1 into cells containing the ΔαYAC, antiviral activity (Figures 9 and 10) and ability of

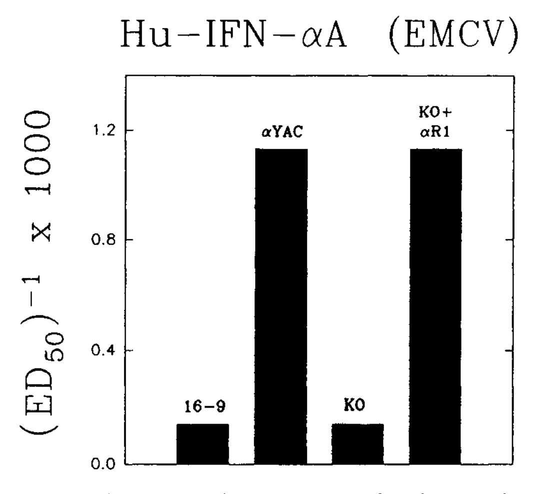


Figure 9. Antiviral Activity of Hu-IFN-αA. The data in the figure represent the reciprocal of the IFN titer (units/mL) for 50% protection of cells (ED_{50}) against EMCV. The 16-9 cell line is a human x hamster hybrid containing the long arm of human Chromosome 6 and a transfected HLA-B7 gene (Cook et al., 1994a; Soh et al., 1993, 1994a); αYAC represents 16-9 cells containing the αYAC (Soh et al., 1994c); KO, 16-9 cells containing the ΔαYAC (αRy10A) with exon II deleted; KO + αR1, represents the KO cells reconstituted by transfection with the Hu-IFN-αR1 cDNA clone. The data for Hu-IFN-αA are shown. Data from Cleary et al. (1994).

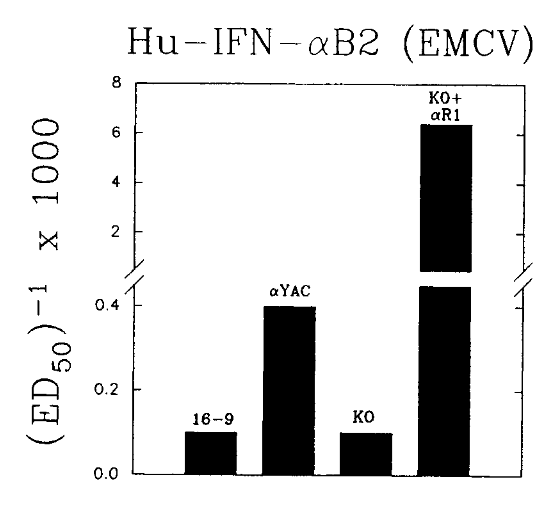


Figure 10. Antiviral activity of Hu-IFN-αB2. The experiments were performed as described in the legend to Figure 9 except that Hu-IFN-αB2 was substituted for Hu-IFN-αA. The data for 16-9 and KO cells are maximal values as the endpoint titer was >10,000 units/ml. Data from Cleary et al. (1994).

Hu-IFN-α to stimulate MHC class I antigens were successfully reconstituted. Thus, the Hu-IFN-aR1 subunit plays a critical role in the functional human Type I IFN receptor complex components of which are encoded on this YAC. In addition, as binding of ligands are retained in the cells containing the YAC with the deletion, it is clear that one or more additional subunits encoded on the YAC are responsible for ligand binding and activity. This system will now allow the identification of additional subunits involved in the response to the Type I IFNs and the functional significance of each.

During our studies describing the αYAC, Novick et al. (1994) reported a second chain now designated, IFN-αR2b, that appears to be another component of the Type I interferon receptor complex. This gene for IFN-αR2b is located on the αYAC and also localizes to the 3x1S region of Chromosome 21. This Hu-IFN-αR2b chain was reported to bind Type I interferons and antibodies to this chain were able to co-immunoprecipitate Jak1. However, expression of neither IFN-αR1 nor IFN-αR2b chains, nor the combination of both, in hamster cells was able to reconstitute functional human receptor activity (Soh et al., 1994b; Mariano et al., 1994; Kotenko and Pestka, unpublished results). In addition, the CHO cells containing the ΔαYAC with the disrupted IFNAR1 gene retained the ability to bind Hu-IFN-αA and Hu-IFN-αB2 (Cleary et al., 1994). Thus, there appears to be a minimum of three components for the

functional Type I interferon receptor: the IFN-αR1 and IFN-αR2b chains, and one or more unidentified chains (see also Uzé et al., 1995). Consistent with this conclusion, a long form of the Hu-IFN-αR2 chain (designatd Hu-IFN-αR2c) was isolated and shown to exhibit activity together with the Hu-IFN-γR1 chain (Lutfalla et al., 1995; Domanski et al., 1995).

The data show that hamster cells containing the αYAC exhibit the properties expected for a functional Type I human IFN receptor complex. The YAC provides genes which are necessary and sufficient to encode this functional Type I interferon receptor complex as measured by three distinct biological assays. Also the specific binding of both [^{32}P]Hu-IFN-αA and [^{32}P]Hu-IFN-αB2 to hamster cells fused to this αYAC demonstrated that the biological functions induced by these IFNs were reflected in the interaction between the ligand and receptor complex. Since this αYAC contains the entire gene for the cloned Hu-IFN-αR1 receptor subunit (~30 kb) and for the Hu-IFN-αR2 chain (Soh et al., 1994c; Cleary et al., 1994; Emanuel and Pestka, unpublished observations), very probably there are other genes responsible for the formation of the receptor complex present in this αYAC. Nevertheless, it is also possible that alternatively-spliced mRNA from the same gene may encode another subunit (Cleary et al., 1992). The results suggest that a high-affinity receptor is composed of the cloned Hu-IFN-αR1 and Hu-IFN-αR2c receptor subunits encoded by genes within this αYAC.

X. RELATIONSHIP TO DOWN'S SYNDROME, GENETICS OF CHROMOSOME 21

Human Chromosome 21 is the smallest and one of the best characterized human chromosomes. Genetic diseases such as Down's syndrome and Alzheimer's disease are associated with this chromosome (Cox and Shimizu, 1990). Even though overlapping clones spanning the entire human Chromosome 21 will facilitate our understanding of the structure of this chromosome (Chumakov et al., 1992b), the methods to identify the function of genes and isolated cDNAs encoded in YACs need to be improved (Duyk et al., 1990; Elvin et al., 1990; Lovett et al., 1991). This summary as well as our previous reports (Soh et al., 1993; Cook et al., 1994) demonstrate that expression of YAC clones in eukaryotic cells can be used to identify the function of genes in YACs as long as genetic

linkage maps and/or cytogenetic studies provide information to permit selection of appropriate YAC clones. If the efficiency of YAC fusion to cells could be increased dramatically, then it might be possible to analyze YAC libraries as cosmid and phage libraries by selecting or screening for specific expressed markers.

The gene encoding the Hu-IFN-γR2 chain (Hu-IFN-γ receptor accessory factor-1, AF-1; Table 1) required for class I MHC antigen induction was mapped to the 3x1S region (Langer et al., 1990), and further localized within the 540 kb GART D142H8 YAC (Soh et al., 1993). A gene encoding a class II cytokine receptor protein (CRFB4) of unknown function was described and found to be near the gene for the cloned Hu-IFN-αR1 receptor cDNA (Lutfalla et al., 1993). These observations suggest that the 3x1S region is rich in genes encoding proteins involved in cytokine function, including Hu-IFN-αR1, Hu-IFN-αR2, CRFB4, and Hu-IFN-γR2 transmembrane receptor chains.

XI. THE CYTOKINE CLASS II RECEPTOR FAMILY

The IFN-αR1, IFN-αR2c, CRFB4, IFN-γR1, and IFN-γR2 chains are members of the cytokine class II receptor family as described by Bazan (1990a, 1990b) and Thoreau et al. (1991), who proposed that the interferon receptors as well as other receptors for cytokines and some growth factors are composed of two folding domains that comprise the ligand binding site which resides in the crevice between the folds. The primary cytokine-receptor interaction was suggested to involve one face of the ligand while another face of the bound cytokine can interact with accessory binding components. A summary of these domains for the interferon-related receptor components is shown in Figures 11 and 12. These homologies relate the interferon receptor components to the fibronectin type III structure, which in turn relates all these structures to the immunoglobulin superfamily.

XII. SIGNAL TRANSDUCTION

Signal transduction through the Type I interferon receptor complex is illustrated in Figure 13. As previously noted, there are likely to be three or more chains of the receptor. Ligand binding seems to be a function of

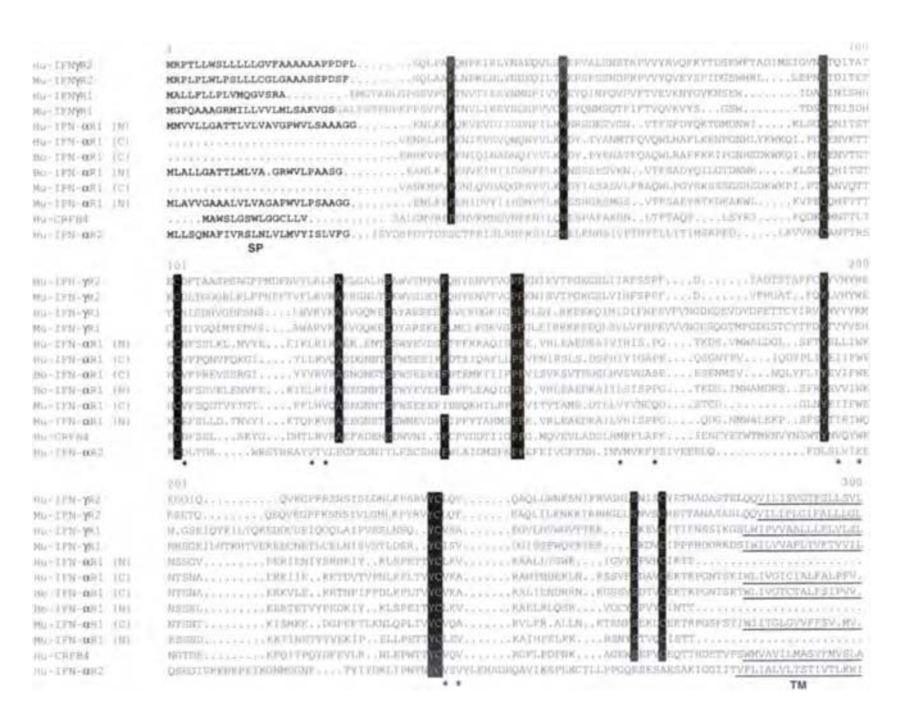


Figure 11. Amino acid sequence alignment of interferon receptors and some members of the class 2 cytokine receptor family. Alignments were made by the algorithm of Needleman and Wunsch (1970) with a gap weight of 5.0 and length weight of 0.3 for the parameters used in the computations. The single extracellular domains of the Hu-IFN-γR2, Mu-IFN-γR2, human and mouse IFN-γ receptors, the human CRF2-4 and the N- and C-terminal repeated domains of the human, mouse and bovine IFN-α receptors are aligned. The following are the abbreviations of the sequences aligned with the Hu-IFN-γR2 (AF-1) protein: Hu-IFN-γR1 (Aguet et al., 1988), Mu-IFN-γR1 (Kumar et al., 1989), CRF2-4 (Lutfalla et al., 1993), Hu-IFN-αR(N) and Hu-IFN-αR(C) (Uzé et al., 1990), Mu-IFN-αR(N) and Mu-IFN-αR(C) (Uzé et al., 1992) and Bo-IFN-αR(N) and Bo-IFN-αR(C) (Mounchel-Vielh et al., 1992; Lim and Langer, 1993); Hu-IFN-αR2 (Novick et al., 1994). The αR(N) and αR(C) designate amino and carboxy terminal domains of the extracellular region, respectively. The highly-conserved cysteines, prolines, charged and aromatic amino acids are boxed. The conserved aromatic and hydroprobic clusters are indicated by an asterisk. The putative signal peptide (SP) and transmembrane domains (TM) are in boldface and are underlined, respectively.

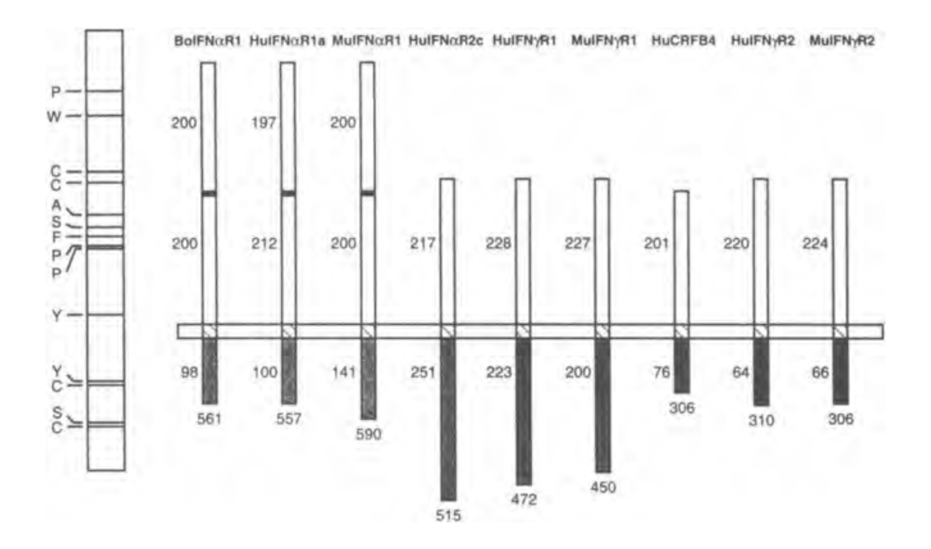
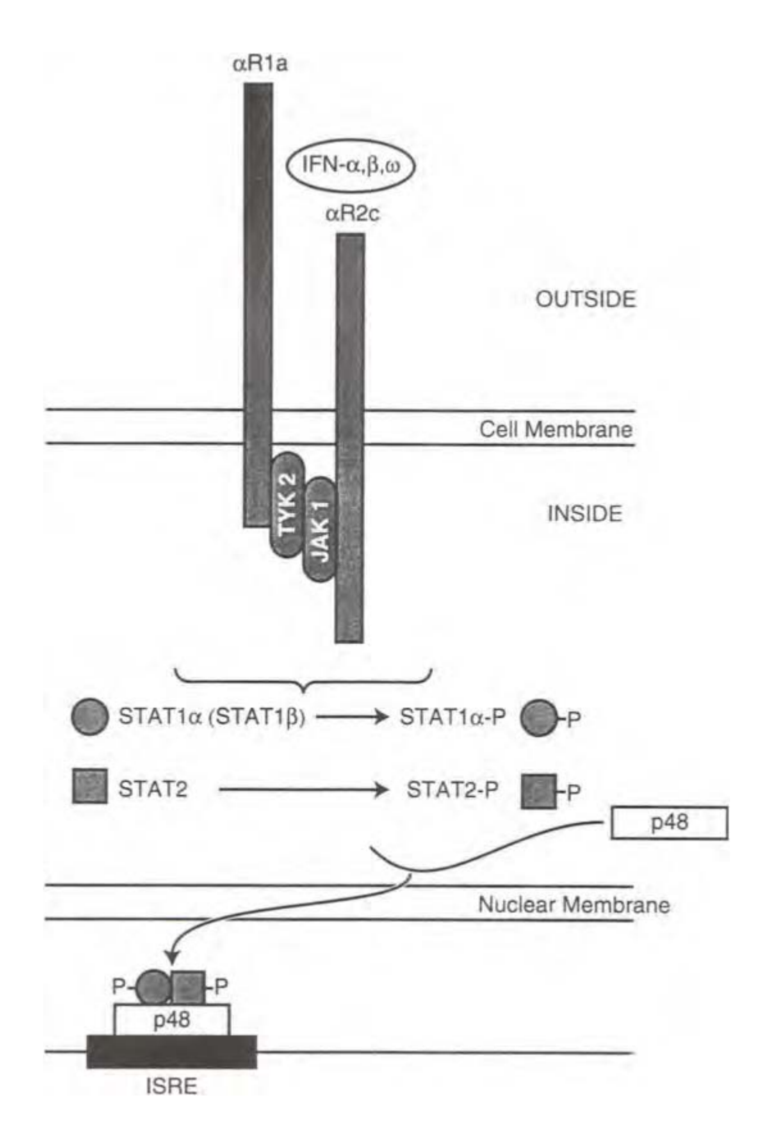


Figure 12. Schematic illustration of cytokine class II receptor family homology with interferon receptors.

both the Hu-IFN-αR1 and Hu-IFN-αR2 chains. Two kinases of the JAK tyrosine kinase family were demonstrated to be necessary for signal transduction: Tyk2 and Jak1 (Pellegrini et al., 1989; John et al., 1991; Velazquez et al., 1992; Pellegrini and Schindler, 1993; Müller et al., 1993). The Tyk2 kinase is activated by binding of the interferon ligands (IFN-α and IFN-β) to cells; and this kinase interacts with the intracellular domain of the IFN-αR1 chain of the receptor (Barbieri et al., 1994; Colamonici et al., 1994a, 1994b). The Jak1 kinase was reported to interact with the IFN-αR2 chain (Novick et al., 1994). Two downstream transcription factors (STAT1 and STAT2) are activated and phosphorylated in response to Type I interferons (Schindler et al., 1992; Larner et al., 1993; Sadowski et al., 1993; Darnell et al., 1995). Once phosphorylated, the STAT1 and STAT2 proteins form a heterodimer, associate with a third cytosolic protein, p48, then translocate to the nucleus where they bind to genes containing an ISRE (the Type I interferon specific regulatory element) as shown in Figure 13.

TYPE I INTERFERON RECEPTOR



IFN-α,β,ω Induced Gene

Figure 13. Illustration of the Type I interferon receptor complex and signal transduction.

ACKNOWLEDGMENTS

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END NOTE

The reconstitution of the panoply of Type I IFN receptor activities not previously obtained with discrete genomic or cDNA clones provides the foundation to isolate the individual components involved. Our use of functional YAC selection and screening (FYSS) to identify this complex receptor is illustrative of the power of this procedure that previously permitted us to identify the Hu-IFN-γR2 chain required for IFN-γ receptor function (Soh et al., 1993, 1994a; Cook et al., 1994). This FYSS technology provides a new dimension for the use of large DNA inserts in identification, characterization, and localization of genes. The beginning of the elucidation of the Type I interferon receptor is just starting with many surprises undoubtedly to come.

REFERENCES

Abramovich, C., Shulman, L.M., Ratovitski, E., Harroch, S., Tovey, M., Eid, P., & Revel, M. (1994). Differential tyrosine phosphorylation of the IFNAR chain of the type I interferon receptor and of an associated surface protein in response to IFN-α and IFN-β. EMBO J. 13, 5871-5877.

Aguet, M. (1980). High-affinity binding of ^{125} I-labeled mouse interferon to a specific cell surface receptor. Nature (London) 284, 768-770.

Aguet, M., Dembic, Z., & Merlin, G. (1988). Molecular cloning and expression of the human interferon-γ receptor. Cell 55, 273-280.

Barbieri, G., Velazquez, L., Schrobogna, M., Fellous, M., & Pellegrini, S. (1994). Activation of the Protein kinase tyk2 by interferon α/β. Eur. J. Biochem. 223, 427-435.

Bazan, J.F. (1990a). Shared architecture of hormone binding domains in type I and II interferon receptors. Cell 61, 753-754.

Bazan, J.F. (1990b). Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA 87, 6934-6938.

Benoit, P., Maguire, D., Plavec, I., Kocher, H., Tovey, M., & Meyer, F. (1993). A monoclonal antibody to recombinant human IFN-α receptor inhibits biologic activity of several species of human IFN-α, IFN-β, and IFN-ω: Detection of heterogeneity of the cellular type I IFN receptor. J. Immunol. 150, 707-716.

Branca, A.A., & Baglioni, C. (1981). Evidence that type I and II interferons have different receptors. Nature (London) 294, 768-770.

Burke, D., Carle, G.F., & Olson, M.V. (1987). Cloning of large segment of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236, 806-812.

Chumakov, I., Rigault, P., Guillou, S., Ougen, P., Billaut, A., Guasconi, G., Gervy, P., Le Gall, I., Soularue, P., Grinas, L., Bougueleret, L., Bellann,-Chantelot, C.,

Lacroix, B., Barillot, E., Gesnouin, P., Pook, S., Vaysseix, G., Frelat, G., Schmitz, A., Sambucy, J.-L., Bosch, A., Estivill, X., Weissenbach, J., Vignal, A., Riethman, H., Cox, D., Patterson, D., Gardiner, K., Hattori, M., Sakaki, Y., Ichikawa, H., Ohki, M., Paslier, L.D., Heilig, R., Antonarakis, S., & Cohen, D. (1992a). Continuum of overlapping clones spanning the entire human chromosome 21q. Nature 359, 380-386.

Chumakov, I.M., Le Gall, I., Billault, A., Ougen, P., Soularue, P., Guillou, S., Rigault, P., Bui, H., De Tand, M.F., Barillot, E., Abderrahim, H., Cherif, D., Berger, R., Le Paslier, D., & Cohen, D. (1992b). Isolation of chromosome 21-specific yeast artificial chromosome from a total human genome library. Nature Genet. 1, 222-225.

Cleary, C.M., Donnelly, R.J., & Pestka, S. (1992). Cloning of an alternatively spliced form of the human interferon alpha receptor. J. Interferon Res. 12, S220.

Cleary, C.M., Donnelly, R.J., Soh, J., Mariano, T.M., & Pestka, S. (1994). Knockout and reconstitution of a functional human type I receptor complex. J. Biol. Chem. 269, 18747-18749.

Cofano, F., Moore, S.K., Tanaka, S., Yuhki, N., Landolfo, S., & Appella, E. (1990). Affinity purification, peptide analysis and cDNA sequence of the mouse interferon-γ receptor. J. Biol. Chem. 265, 4064-4071.

Colamonici, O.R., & Domanski, P. (1993). Identification of a novel subunit of the type I interferon receptor localized to human chromosome 21. J. Biol. Chem. 268, 10895-10899.

Colamonici, O.R., & Pfeffer, L.M. (1991). Structure of the human interferon alpha receptor. Pharmacology & Therapeutics 52, 227-233.

Colamonici, O.R., D'Alessandro, F. Diaz, M.O., Gregory, S.A., Neckers, L.M., & Nordan, R. (1990). Characterization of three monoclonal antibodies that recognize the interferon alpha 2 receptor. Proc. Natl. Acad. Sci. USA 87, 7230-7234.

Colamonici, O.R., Pfeffer, L.M., D'Alessandro, F., Platanias, L.C., Gregory, S.A., Rosolen, A., Nordan, R., Cruciani, R.A., & Diaz, M.O. (1992). Multichain structure of the IFN-alpha receptor on hematopoietic cells. J. Immunol. 148, 2126-2132.

Colamonici, O.R., Porterfield, B., Domanski, P., Constantinescu, S., & Pfeffer, L.M. (1994a). Complementation of the interferon α response in resistant cells by expression of the cloned subunit of the interferon αII receptor. J. Biol. Chem. 269, 9598-9602.

Colamonici, O.R., Uyttendaele, H., Domanski, P., Yan, H., & Krolewski, J.J. (1994b). $p135^{Tyk2}$, an interferon-α-activated tyrosine kinase, is physically associated with an interferon-α receptor. J. Biol. Chem. 269, 3518-3522.

Constantinescu S.N., Croze, E., Wang, C., Murti, A., Basu, L., Mullersman, J.E., & Pfeffer, L.M. (1994). Role of interferon alpha/beta receptor chain 1 in the structure and transmembrane signaling of the interferon alpha/beta receptor complex. Proc. Natl. Acad. Sci. USA 91, 9602-9606.

Cook, J.R., Emanuel, S.L., & Pestka, S. (1993). Yeast artificial chromosome fragmentation vectors that utilize $URA3^+$ selection. Gene Analysis: Techniques & Applic. 10, 109-112.

Cook, J.R., Jung, V., Schwartz, B., Wang, P., & Pestka, S. (1992). Structural analysis of the human interferon-gamma receptor: Specific requirement of a small segment

of the intracellular domain for class I MHC antigen induction and antiviral activity. Proc. Natl. Acad. Sci. USA 89, 11317-11321.

Cook, J.R., Emanuel, S.L., Donnelly, R.J., Soh, J., Mariano, T.M., Schwartz, B., Rhee, S., & Pestka, S. (1994). Sublocalization of the human interferon-gamma receptor accessory factor gene and characterization of accessory factor activity by yeast artificial chromosomal fragmentation. J. Biol. Chem. 269, 7013-7018.

Cox, D.R., & Shimizu, N. (1990). Report of the committee on the genetic constitution of chromosome 21. Cytogenet. Cell Genet. 58, 235-244.

Darnell, J.E., Kerr, I.M., & Stark, G.R. (1995). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., & Colamonici, O.R. (1995). Cloning and expression of a long form of the β subunit of the interferon αβ receptor that is required for signaling. J. Biol. Chem. 270, 21606-21611.

Duyk, G.M., Kim, S., Myers, R.M., & Cox, D.R. (1990). Exon trapping: A genetic screen to identify candidate transcribed sequences in cloned mammalian genomic DNA. Proc. Natl. Acad. Sci. USA 87, 8995-8999.

Elvin, P., Slynn, G., Black, D., Graham, A., Butler, R., Riley, J., Anand, R., & Markham, A.F. (1990). Isolation of cDNA clones using yeast artificial chromosome probes. Nucleic Acid. Res. 18, 3913-3917.

Emanuel, S.L., Cook, J.R., O'Rear, J., Rothstein, R., & Pestka, S. (1995). New vectors for manipulation and selection of functional YACs containing human DNA inserts. Gene 155, 167-174.

Epstein, C.J., McManus, N.H., & Epstein, L.B. (1982). Direct evidence that the gene product of the human chromosome 21 locus, IFRC, is the interferon-α receptor. Biochem. Biophy. Res. Comm. 107, 1060-1066.

Flores, I., Mariano, T.M., & Pestka, S. (1991). Human interferon omega (ω) binds to the α/β receptor. J. Biol. Chem. 266, 19875-19877.

Friedman, R.M. (1967). Interferon binding: The first step in establishment of antiviral activity. Science 156, 1760-1761.

Gray, P.W., Leong, S., Fennie, E.H., Farrar, M.A., Pingel, J.T., Fernandez-Luna, J., & Schreiber, R.D. (1989). Cloning and expression of the cDNA for the murine interferon-γ receptor. Proc. Natl. Acad. Sci. USA 86, 8497-8501.

Hannigan, G.E., Lau, A.S., & Williams, B.R. (1986). Differential human interferon alpha receptor expression on proliferating and non-proliferating cells. Eur. J. Biochem. 157, 187-193.

Hemmi, S., Peghini, P., Metzler, M., Merlin, G., Dembic, Z., & Aguet, M. (1989). Cloning of Murine interferon-γ receptor cDNA: Expression in human cells mediates high affinity binding but is not sufficient to confer sensitivity to murine interferon-γ. Proc. Natl. Acad. Sci. USA 86, 9901-9905.

Hibino, Y., Mariano, T.M., Kumar, C.S., Kozak, C.A., & Pestka, S. (1991). Expression and reconstitution of a biologically active mouse interferon gamma receptor in hamster cells: Chromosome location of an accessory factor. J. Biol. Chem. 266, 6948-6951.

Hibino, Y., Kumar, C.S., Mariano, T.M., Lai, D., & Pestka, S. (1992). Chimeric interferon gamma receptors demonstrate an accessory factor required for activity interacts with the extracellular domain. J. Biol. Chem. 267, 3741-3749.

John, J., McKendry, R., Pellegrini, S., Flavell, D., Kerr, I.M., & Stark, G.R. (1991). Isolation and characterization of a new mutant human cell line unresponsive to alpha and beta interferons. Mol. Cell. Biol. 11, 4189-4195.

Jung, V. (1991) The Human Interferon Gamma Receptor and Signal Transduction. Ph.D. dissertation submitted to the Graduate School—New Brunswick, Rutgers, The State University of New Jersey, and the Graduate School of Biomedical Sciences, Robert Wood Johnson Medical School.

Jung, V., & Pestka, S. (1986). Selection and screening of transformed NIH3T3 cells for enhanced sensitivity to human interferons α and β. In: *Methods in Enzymology,* Vol. 119 (Pestka, S., Ed.). Academic Press, New York, pp. 597-611.

Jung, V., Jones, C., Rashidbaigi, A., Geyer, D.D., Morse, H.G., Wright, R.B., & Pestka, S. (1988). Chromosome mapping of biological pathways by fluorescence-activated cell sorting and cell fusion: The human interferon gamma receptor as a model system. Somatic Cell and Molecular Genetics 14, 583-592.

Jung, V., Jones, C., Kumar, C.S., Stefanos, S., O'Connell, S., & Pestka, S. (1990). Expression and reconstitution of a biologically active human interferon gamma receptor in hamster cells. J. Biol. Chem. 265, 1827-1830.

Jung, V., Rashidbaigi, A., Jones, C., Tischfield, J.A., Shows, T.B., & Pestka, S. (1987). Human chromosomes 6 and 21 are required for sensitivity to human interferon gamma. Proc. Natl. Acad. Sci. USA 84, 4151-4155.

Kumar, C.S., Muthukumaran, G., Frost, L.J., Noe, M., Ahn, Y.-H., Mariano, T.M., & Pestka, S. (1989). Molecular characterization of the murine interferon gamma receptor cDNA. J. Biol. Chem. 264, 17939-17946.

Langer, J.A., & Pestka, S. (1988). Interferon receptors. Immunol. Today 9, 393-399.

Langer, J.A., Rashidbaigi, A., Lat, L.-W., Patterson, D., & Jones, C. (1990). Sublocalization on chromosome 21 of human interferon-α receptor gene and the gene for an interferon-γ response protein. Somat. Cell Mol. Genet. 16, 231-240.

Larner, A.C., David, M., Feldman, G.M., Igarashi, K.-I., Hackett, R.H., Webb, D.S.A., Sweitzer, S.M., Petricoin, E.F., III, & Finbloom, D.S. (1993). Tyrosine phosphorylation of DNA binding proteins by multiple cytokines. Science 261, 1730-1733.

Lengyel, P. (1982) Biochemistry of interferons and their actions. Ann. Rev. Biochem. 51, 251-282.

Li, J., & Roberts, R. M. (1994). Interferon-τ and interferon-α interact with the same receptors in bovine endometrium. Use of a readily iodinatable form of recombinant interferon-τ for binding studies. J. Biol. Chem. 269, 13544-13550.

Lim, J-K., & Langer, J.A. (1993). Cloning and characterization of a bovine alpha interferon receptor. Biochem. Biophys. Acta 1173, 314-319.

Lim, J.-K., Xiong, J., Carrasco, N., & Langer, J.A. (1994). Intrinsic ligand binding properties of the human and bovine α-interferon receptors. FEBS Lett. 350, 281-286.

Lovett, M., Kere, J., & Hinton, L.M. (1991). Direct selection: A method for the isolation of cDNAs encoded by large genomic regions. Proc. Natl. Acad. Sci. USA 88, 9628-9632.

Lutfalla, G., Roeckel, N., Mogensen, K.E., Mattei, M-G., & Uzé, G. (1990). Assignment of the human interferon-α receptor gene to chromosome 21q22.1 by *in situ* hybridization. J. Interferon Res. 10, 515-517.

Lutfalla, G., Gardiner, K., Proudhon, D., Vielh, E., & Uzé, G. (1992). The structure of the human interferon α/β receptor gene. J. Biol. Chem. 267, 2802-2809.

Lutfalla, G., Gardiner, K., & Uzé, G. (1993). A new member of the cytokine receptor gene family maps on chromosome 21 at less than 35 kb from IFNAR. Genomics 16, 366-373.

Lutfalla, G., Holland, S.J., Cinato, E., Monneron, D., Reboul, J., Rogers, N.C., Smith, J.M., Stark, G.R., Gardiner, K., Mogensen, K.E., Kerr, I.M., & Uzé, G. (1995). Mutant U5A cells are complemented by an interferon-αβ receptor subunit generated by alternative processing of a new member of a cytokine receptor gene cluser. EMBO J. 14, 5100-5108.

Mariano, T.M., Donnelly, R.J., Soh, J., & Pestka, S. (1992). Structure and function of the type I interferon receptor. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D., Dianzani, F., Fleischman, W.R., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Stanton, G.J., & Tyring, S.K., Eds.). University Texas Medical Branch at Galveston, Galveston, TX, pp. 129-138.

Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, Silvennoinen, O., Harpur, A.G., Barbieri, G., Witthuhn, B.A., Schindler, C., Pellegrini, S., Wilks, A.F., Ihle, J.N., Stark, G.R., & Kerr, I.M. (1993). The protein tyrosine kinase JAK1 complements defects in interferon-α/β and-γ signal transduction. Nature 366, 129-135.

Munro, S., & Maniatis, T. (1989). Expression cloning of the murine interferon-γ receptor cDNA. Proc. Natl. Acad. Sci. USA 86, 9248-9252.

Novick, D., Cohen, B., & Rubinstein, M. (1994). The human interferon α/β receptor: Characterization and molecular cloning. Cell 77, 391-400.

Pellegrini, S., & Schindler, C. (1993). Early events in signalling by interferons. Trends Biochem. Sci. 18, 338-342 [Eratum appears Vol. 18, p. 428].

Pellegrini, S., John, J., Shearer, M., Kerr, I.M., & Stark, G.R. (1989). Use of a selectable marker regulated by alpha interferon to obtain invitations in the signaling pathway. Mol. Cell. Biol. 9, 4605-4612.

Pestka, S., Langer, J.A., Zoon, K.C., & Samuel, C.E. (1987). Interferons and their actions. Ann. Rev. Biochem. 56, 727-777.

Rashidbaigi, A., Langer, J.A., Jung, V., Jones, C., Morse, H.G., Tischfield, J., Trill, J.J., Kung, H-F., & Pestka, S. (1986). The gene for the human immune interferon receptor is located on chromosome 6. Proc. Natl. Acad. Sci. USA 83, 384-388.

Raziuddin, A., & Gupta, S.L. (1985). Receptors for human interferon-alpha: two forms of interferon-receptor complexes identified by chemical cross-linking. In: *The 2-5A System: Molecular and Clinical Aspects of the Interferon-Regulated Pathway* (Williams, B.R.G., & Silverman, R.H., Eds.). Alan R. Liss, New York.

Raziuddin, A., Sarkar, F.H., Dutkowski, R., Shulman, L., Ruddle, F.H., & Gupta, S.L. (1984). Receptors for human α and β interferon but not for γ interferon are specified by human chromosome 21. Proc. Natl. Acad. Sci. USA 81, 5504-5508.

Revel, M., Bash, D., & Ruddle, F. H. (1976). Antibodies to a cell-surface component coded by human chromosome 21 inhibit action of interferon. Nature (London) 260, 139-141.

Revel, M., Cohen, B., Abramovich, C., Novick, D., Rubinstein, M., & Shulman, L. (1991). Components of the human type I IFN receptor system. J. Interferon Res. 11 (Suppl.), S61.

Sadowski, H.B., Shuai, K., Darnell, J.E., & Gilman, M.Z. (1993). A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. Science 261, 1739-1744.

Schindler, C., Shuai, K., Prezioso, V.R., & Darnell, J.E. (1992). Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 257, 809-813.

Sen, G.C., & Lengyel, P. (1992). The interferon system. A bird's eye view of its biochemistry. J. Biol. Chem. 267, 5017-5020.

Shulman, L.M., Kamarck, M.E., Slate, D.L., Ruddle, F.H., Branca, A.W., Baglioni, C., Maxwell, B.L., Gutterman, J., Anderson, P., & Nagler, C. (1984). Antibodies to chromosome 21 coded cell surface components block binding of human alpha interferon but not gamma interferon to human cells. Virology 137, 422-427.

Slate, D.L., Shulman, L., Lawrence, J.B., Revel, M., & Ruddle, F.H. (1978). Presence of human chromosome 21 alone is sufficient for hybrid cell sensitivity to human interferon. J. Virol. 25, 319-325.

Soh, J., Donnelly, R.J., Mariano, T.M., Cook, J.R., Schwartz, B., & Pestka, S. (1993). Identification of a yeast artificial chromosome clone encoding an accessory factor for the human interferon gamma receptor: Evidence for multiple accessory factors. Proc. Natl. Acad. Sci. USA 90, 8737-8741.

Soh, J., Donnelly, R.J., Kotenko, S., Mariano, T.M., Cook, J.R., Wang, N., Emanuel, S.L., Schwartz, B., Miki, T., & Pestka, S. (1994a). Identification and sequence of an accessory factor required for activation of the human interferon gamma receptor. Cell 76, 793-802.

Soh, J., Mariano, T.M., Bradshaw, G., Donnelly, R.J., & Pestka, S. (1994b). Generation of random internal deletion derivatives of YACs by homologous targeting to *alu* sequences. DNA & Cell Biol. 13, 301-309.

Soh, J., Mariano, T.M., Lim, J-K., Izotova, L., Mirochnitchenko, O., Schwartz, B., Langer, J., & Pestka, S. (1994c). Expression of a functional human type I interferon receptor in hamster cells: Application of functional YAC screening. J. Biol. Chem. 269, 18102-18110.

Tan, Y.H., Tischfield, J., & Ruddle, F.H. (1973). The linkage of genes for the human interferon-induced antiviral protein and indophenol oxidase-B tarits to chromosome G-21. J. Exp. Med. 137, 317-330.

Thoreau, E., Petridou, B., Kelly, P.A., Djiane, J., & Mornon, J.P. (1991). Structural symmetry of the extracellular domain of the cytokine/growth hormone/prolactin receptor family and interferon receptors revealed by hydrophobic cluster analysis. FEBS Lett. 282, 26-31.

Uzé, G., Lutfalla, G., & Gresser, I. (1990). Genetic transfer of a functional human interferon-α receptor into mouse cells: Cloning and expression of its cDNA. Cell 60, 225-234.

Uzé, G., Lutfalla, G., Bandu, M-T., Prodhon, D., & Mogensen, K. (1992). Behavior of a cloned murine interferon α/β receptor expressed in homospecific or heterospecific background. Proc. Natl. Acad. Sci. USA 89, 4774-4778.

Uzé, G., Lutfalla, G., Eid, P., Maury, C., Bandu, M.T., Gresser, I., & Mogensen, K. (1991). Murine tumor cells expressing the gene for the human interferon alpha beta receptor elicit antibodies in syngeneic mice to the active form of the receptor. Eur. J. Immunol. 21, 447-451.

Uzé, G., Lutfalla, G., & Mogenson, K.E. (1995). α and β interferons and their receptor and their friends and relatives. J. Interferon Cytokine Res. 15, 3-26.

Vanden Broecke, C., & Pfeffer, L.M. (1988). Characterization of interferon-alpha binding sites on human cell lines. J. Interferon Res. 8, 803-811.

Velazquez, L., Fellous, M., Stark, G.R., & Pellegrini, S. (1992). A protein tyrosine kinase in the interferon alpha/beta signaling pathway. Cell 70, 313-322.

Whaley, A.E., Meka, C.S., Harbison, L.A., Hunt, J.S., & Imakawa, K. (1994). Identification and cellular localization of unique interferon mRNA from human placenta. J. Biol. Chem. 269, 10864-10868.

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INTERFERON-BETA:

STRUCTURE, DIFFERENTIAL ACTIONS, AND MEDICAL APPLICATIONS

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I. MOLECULAR STRUCTURE OF IFN-β

IFN-β produced by human fibroblasts in response to viruses or double stranded (ds) RNA inducers (see Section IV) is a protein antigenically distinct from the other group of type I Interferons, the IFN-α subtypes (Havell et al., 1975; Hayes, 1981). Purification of this natural IFN-β indicated a specific NH2-terminal amino acid sequence (Knight et al.,1980; Stein et al.,1980). cDNA cloning of IFN-β 11S mRNA was achieved by differential hybridization (Taniguchi et al., 1980b; Weissenbach et al., 1980). The entire amino-acid sequence of mature IFN-β deduced from the cDNA comprises 166 aminoacids preceded by 21 signal peptide residues, and is about 30% homologous to IFN-α subtypes (Taniguchi et al., 1980a, 1980b). IFN-β is richer in hydrophobic residues (39.8%) than IFN-α (35%). The mature IFN-β sequence (Figure 1) was

```
         ____10____A____20____         30          40          50      ______60
IFN-β   MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIY
IFN-α2    CDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFPQEEF-GNQFQKAETIPVLH
"αcon     CDLPQTHSLGNRRTLILLAQMRRISPFSCLKDRHDFGFPQEEFDGNQFQKAQAISYLH
        _B_____70          80 ___C___90_       100         110       __120
IFN-β   EMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSL
IFN-α2  EMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKEDSIL
"αcon   EMIQQTFNLFSTKDSSAAWDESLLEKFSTELYQQLNDLEACVIQEVGVEETPLMNEDSIL
        _D_____130____      140 ___E___150_________160
IFN-β   HLKRYYGRILHYLKAKEYSHCAWTIVAVEILRNFYFINRLTGYLRN
IFN-α2  AVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE
"αcon   AVRKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSFSTNLQKRLRRKD
```

IFN-β N^{80}-Carbohydrate: Biantennary:

```
                      {Galβ1->4GlcNAcβ1->2Manα1\6
[Siaα2->3(6)]0-2{                                      Manβ1->4R,R'
                      {Galβ1->4GlcNAcβ1->2Manα1/3
```

Triantennary:

```
                      {Galβ1->4GlcNAcβ1 \6(4)
[Siaα2->3(6)]n  {                      Manα1\6(3)
                      {Galβ1->4GlcNacβ1 /2          Manβ1->4R,R'
                      {Galβ1->4GlcNAcβ1->2Manα1/3(6)
```

Figure 1. Top: Amino acid sequence of mature human IFN-β aligned with that of IFN-α2 and IFN-α consensus. The five helices A-E are overlined (see Figure 2a). Bottom: structure of carbohydrate chains attached to asparagine-80 in human glycosylated IFN-β.

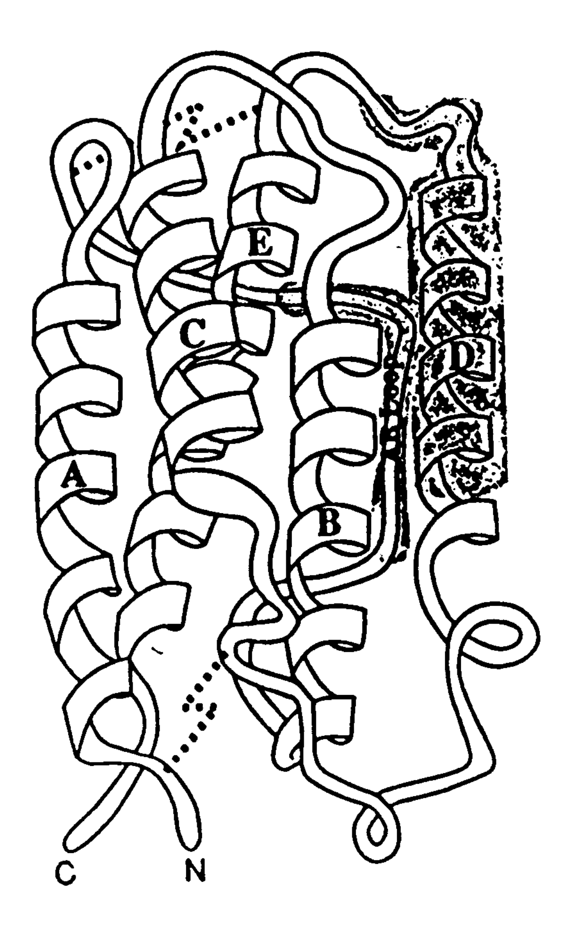


Figure 2A. Schematic drawing of the structure of murine IFN-β derived from crystallographic data (see Section I).

confirmed by protein sequencing of natural fibroblast IFN (Hosoi et al., 1988). Natural human IFN-β is a glycoprotein of 22-24 kDa (Knight and Fahey, 1982); the structure of the complex and somewhat heterogenously charged carbohydrate moities linked to Asn80 (82% biantennary and 18% triantennary, Figure 1) was elucidated for natural IFN-β, and no O-linked sugars were found (Kagawa et al., 1988; Utsumi et al., 1989). Ungycosylated IFN-β produced in *E.coli* is active but less stable (Derynck et al., 1980; Mark et al., 1984) and deglycosylated fibroblast IFN-β has antigrowth and antiviral activity, although reduced (Knight and Fahey, 1982). The 18 kDa *E. Coli*-produced IFN-β is more hydrophobic and basic than the natural IFN-β (Utsumi et al., 1987) and differs

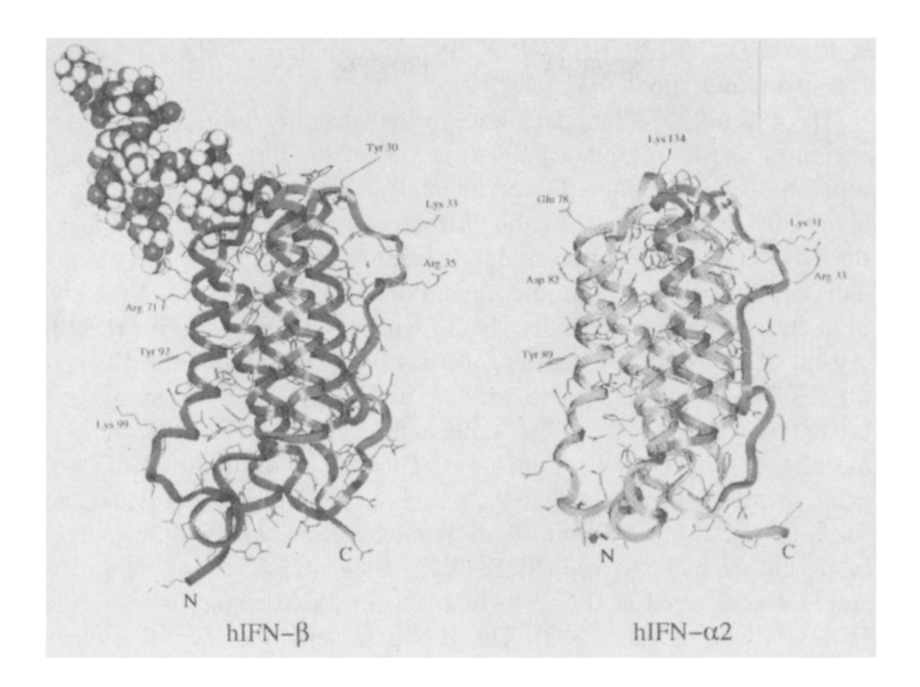


Figure 2B. Computer theoretical models of human IFN-β and human IFN-α2 calculated from coordinates of the murine IFN-β crystallographic structures (courtesy of N. El Tayar, A. Ythier, and Ares-Serono).

immunologically (Colby et al., 1984). Since the carbohydrates may reduce antigenicity in man, care was taken to produce recombinant human IFN-β in mammalian cells such as Chinese Hamster Ovary (CHO) cells which secrete it with sugar structures closest to those of natural IFN-β (Utsumi et al., 1989) and with high antiviral specific activities of 4- 5 x10^8 units per mg protein similar to natural IFN-β (Chernajovsky et al., 1984).

The three-dimensional structure of an IFN-β has been determined by X-ray crystallography of a murine recombinant IFN-β produced in *E.coli* (Senda et al.,1992). Five alpha helical domains were observed (A-E, Figure 2a) separated by intervening loops of which the AB loop is the longest. Although unique, this structure is close to that of growth hormone (DeVos et al.,1992) and of other cytokines in that the core structure appears to be a bundle of four alpha helices (A,B,C, and E), the region forming the CE (or 3-4) loop being itself folded into helix D (Senda et al., 1992). The position of the helices are: A, 6-23; B, 50-65,

C, 78-87; D,112-129; E,137-152; the corresponding residues in human IFN-β are underlined in Figure 1.

The murine rIFN-β model allows us to make only limited predictions regarding the three-dimensional structure of the human IFN-β which remains to be determined experimentally since the two species have about 50% homology. A notable difference is that murine IFN-β lacks the Cys^{31}-Cys^{141} disulfide bridge present in human IFN-β (Figures 1 and 2a): this bridge would join the middle of the AB loop with the end of the DE loop which are indeed in close proximity in the murine rIFN-β crystal structure supporting the general validity of the model (Mitsui et al., 1993). This bridge, also present in HuIFN-αs, is important for bioactivity (Senda et al., 1992; Edge et al., 1986). HuIFN-β has a third cysteine residue, which is unpaired. This Cys^{17} residue in HuIFN-β causes abnormal folding when the protein is produced in *E.coli* and had to be mutated into a Serine to allow bacterial production, the mutein being known as betaseron (Mark et al., 1984). Cys^{17} is among the 16 residues conserved in IFN-β of many species and which differ in the IFN-αs (Senda et al., 1992). The IFN-β proteins lack two Cysteines forming the 1-99 S-S bridge in IFN-α, but this missing bridge (Figure 2) is not required for bioactivity since truncation of the 4 aminoterminal residues of IFNs leaves active molecules. Based on a review of various gene manipulations such as hybrid scanning and site-directed mutagenesis, Senda et al (1992) proposed that the conserved active sites of IFN-β and IFN-α are most likely to be in the AB loop, residues 31-37 (with Leu32 and Arg35 as functionally important), in the D helix residues 123-126 (with Tyr125) and in the start of the DE loop, residues 136,137 (numbered as in Figure 1). These sites are neighboring in the crystal structure model (Figure 2a, shaded area).

Differences between the IFN-β and IFN-α structures were analyzed by Fish (1992) who pointed out that an exposed region 81-98 (Figure 1; 78-95 in their numbering) shows sequence divergence in IFN-β. This region corresponding to helix C and beginning of the CD loop, could be an active site functioning in addition to the main active sites in loop AB and helix D (Figure 2a). Mutations in helix C affect species-specific recognition of some IFNs (DiMarco et al., 1994). The murine IFN-β crystallographic parameters were used to predict the three-dimensional structure of a "consensus" IFN-α and superimposition of the two structures supports the non-identity of IFN-β and IFN-α (Korn et al.,1994). Differences were evident in the CD loop folding and position of the

juxtaposed carboxy- and amino-terminals, but since their truncation or the deletion of residues corresponding to 104-113 (in Figure 1) within the CD loop of IFN-α2 leave active molecules (Edge et al., 1986), the more relevant differences were again ascribed to the exposed residues 81-98 (of Figure 1) within helix C (Asn86, Leu88, Ala89, Asn 90) and beginning of CD loop (His97, Lys99), these IFN-β residues being non-homologous in all IFN-αs. To better compare the human IFN-β to a human IFN-α2 subtype, models for the two molecules were predicted on the basis of the crystallographic murine IFN-β data (El Tayar and Ythier, personal communication, Figure 2b). The AB loop and D helix (in red) show conserved structure and common residues (although with some variations) in the IFN-β and IFN-α2. At the left, the C helix and beginning of CD loop show conformation differences in particular around Lys99. The putative carbohydrate structure of glycosylated IFN-β also bulges on this side of the molecule, further stressing the difference with IFN-α2. The models suggest that from this side, topologically opposite to the common active site, IFN-β would look quite different from IFN-α and have certain differences in its mode of interaction with the receptor system.

II. RECEPTOR BINDING AND SIGNAL TRANSDUCTION

The results of the structural studies and structure-activity analysis of IFN-β summarized previously, in particular the suggestion of multisite interactions of IFN regions D, AB as well as C with the receptor (Korn et al., 1994; Uze et al.,1995), are relevant to the mechanism by which IFN-β may differ from IFN-α in its action through the receptor (see also next section). Since competition can be readily observed between IFN-β and IFN-αs for binding to cellular receptor sites, it is assumed that both subtypes interact with closely related, if not identical, domains of a type I IFN Receptor system (Branca and Baglioni,1981; O'Rourke et al., 1984; Merlin et al., 1985; Pestka et al., 1987). However, binding affinities may vary between subtypes, IFN-β showing often higher affinity (Ruzicka et al., 1987; Johns et al., 1992). The type I IFN receptor system is encoded by chromosome 21 (Revel et al., 1976; Raziuddin et al., 1984) but a number of different genes appear involved in IFN binding (in 21q22.1; Soh et al., 1994) and signal transduction (in 21q22.2-3, Hertzog et al., 1994). Until now, two components of the type I IFN receptor have been cloned. One component IFNAR1 (see Uze et al., 1990, 1995 for review) is a 105-110 kDa transmembrane protein whose extracellular

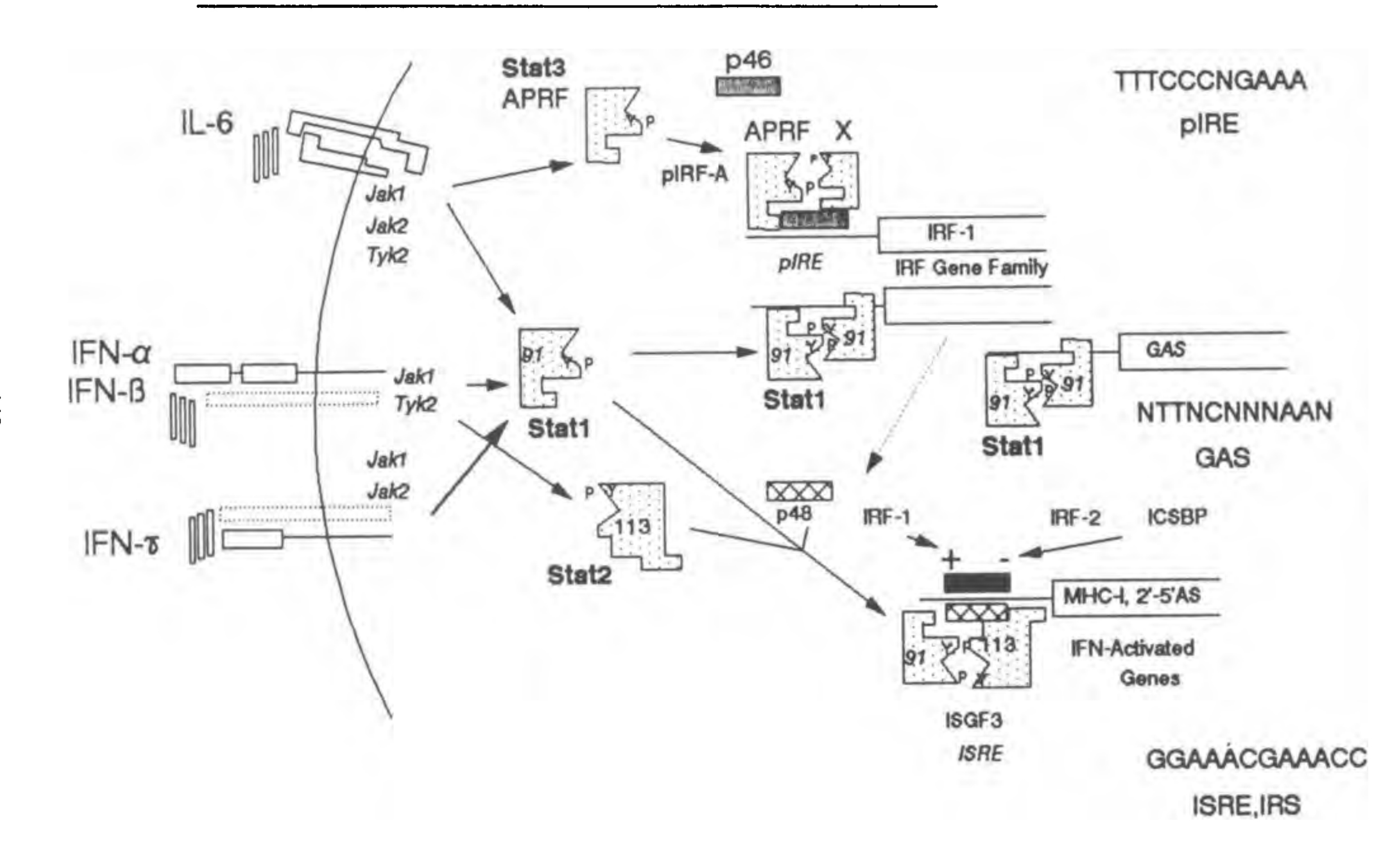
part has two ligand-binding domains, both conforming to the class II cytokine receptor family (Bazan, 1990). There is good evidence that the IFNAR1 protein is involved in the binding and action of IFN-α subtypes (α2,8, or ω) as well as of IFN-β, as shown by neutralizing monoclonal antibodies to IFNAR1 (Benoit et al., 1993) and by knock-out of the IFNAR1 gene in a yeast artificial chromosome (YAC) clone in CHO cells (Cleary et al., 1994). Transfection of the human IFNAR1 cDNA into murine cells elicits selective binding and response to the human IFN-α8 subtype but not α2 or β (Uze et al., 1990,1995). However, transfection into hamster cells elicits a response to human IFN-β but none to IFN-α8 (Abramovich et al.,1994a). This discrimination between IFN-β and IFN-α (see Section IIIA) probably results from the fact that IFNAR1 acts in conjunction with other components contributed to these transfections by the host cell. Glycosphingolipids on IFNAR1 could modulate its activity (Cohen et al., 1987; Lingwood and Shiu, 1992; Ghislain et al., 1994). A second protein component of the human IFN-α,β receptor, IFNABR, or IFNAR2 was found as a secreted protein and cloned as a 51 kDa transmembrane protein with one domain of the class II cytokine receptor type (Novick et al., 1994). IFNAR2 has intrinsic binding activity for various human IFN-α subtypes and for IFN-β but does not produce an IFN response because of its short intracytoplasmic (IC) domain. Resulting from a different gene splicing (Lutfalla et al., 1995), the active 95-100 kDa IFNAR2-2 (or 2-c) molecule has a long IC domain and elicits signal transduction in response to all human IFN subtypes when transfected along with IFNAR1. The receptor subunits α and β defined by monoclonal antibodies (Colamonici et al., 1992; Colamonici and Domanski, 1993) correspond to IFNAR1 and IFNAR2-2 (Domanski et al., 1995). The IFNAR2-2 gene is close to that of IFNAR1 (Soh et al., 1994; Lutfalla et al., 1995), but there may still be additional gene products from chromosome 21 which are needed for the assembly of the type I IFN receptor complex which is fully active in eliciting all biological responses to the IFN-α subtypes and to IFN-β (Herzog et al., 1994).

In addition to the common binding sites on IFNAR1 and IFNAR2 for which IFN-α and IFN-β compete, there is evidence for specific interactions of IFN-β which differ from those of the IFN-α subtypes. Notably, a membranal 95 kDa protein βPTyr or BRAP is found associated with IFNAR1 as a tyrosine phosphorylated protein which is immuno-precipitated by anti-IFNAR1 antibodies only in response to IFN-β (Abramovich et al., 1994b; Platanias et al., 1994). This component is not seen associ-

ated with IFNAR1 after treatment with a variety of IFN-α subtypes including α2, α8, or ω. Theoretically, this βPTyr component could interact through its extracellular domain with a specific site on IFN-β (helix C, CD?), separate from the common binding site on which IFN-α and IFN-β compete for IFNAR1,2 and which would involve the more homologous regions of IFN-α and IFN-β (helix D and loop AB, see Section I, Figure 2). It is not excluded that βPtyr could be a receptor chain associated with IFNAR1 and rapidly tyrosine phosphorylated only when IFN-β interacts with the cell. However, recent evidence shows that IFNAR1 and IFNAR2-2, which are both tyrosine phosphorylated in response to IFN-α and IFN-β, form a stable complex which can be immunoprecipitated by either anti-IFNAR1 or anti-IFNAR2 only when IFN-β is the ligand but not when IFN-α subtypes are used (Platanias et al., 1996). The βPTyr component would then be IFNAR2-2 but this IFN-β specific effect shows that IFN-β has a distinct interchain binding and signaling action on the receptor system it shares with IFN-α. This could explain many of the different activities of IFN-β on cells (see Section II).

Tyrosine phosphorylations are early key events in receptor signaling and for the type I IFN receptor system two cytoplasmic tyrosine kinases were shown to be required by analysis of defective mutants: Tyk2 (Velazquez et al., 1992) and Jak1 (Müller et al., 1993). Such kinases of the Janus family (Jak1,Jak2,Tyk2) have multiple functions in the action of IFN-α,β, IFN-γ, IL-6, and other cytokines (Pellegrini and Schindler, 1993; Kishimoto et al., 1994; Darnell et al., 1994). In the type I IFN receptor, the IFNAR1 chain is tyrosine phosphorylated within minutes of adding IFN-α or IFN-α, and Tyk2 is found constitutively associated with IFNAR1 on its intracytoplasmatic domain (Colamonici et al., 1994; Abramovich et al., 1994b). Instead, the Jak1 kinase is bound to the IFNABR/IFNAR2 chain (Novick et al., 1994), the long form of which is the 100 kDa receptor β subunit that is tyrosine phosphorylated along with the α- chain (IFNAR1) in response to both IFN-α and IFN-β (Platanias and Colamonici, 1992; Platanias et al., 1994). As in the case of the IFN-γ receptor (Greenlund et al., 1994), the phosphorylation of specific tyrosines in the intracytoplasmatic domains of the type I IFN Receptor chains may be directly involved in binding of Stat factors (signal transducers and activators of transcription, see Darnell et al., 1994 for review) through their sarc-homology (SH2) domains. The Stat1(p91) and Stat2 (p113) transcription factors (Figure 3), once tyrosine phosphorylated, migrate to the nucleus where they combine with ISGF3γ (p48)

ACTIVATION OF STAT91 AND OTHER TYR-P FACTORS BY IL-6 AND IFNs



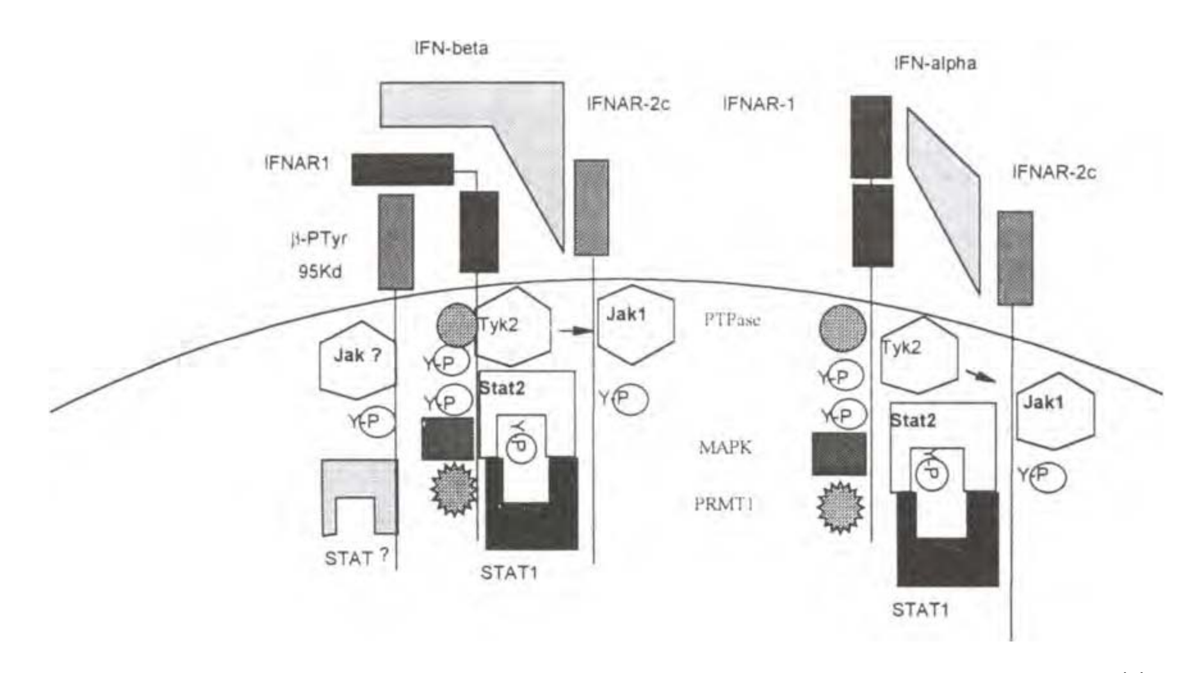


Figure 3. (A) Signal transduction pathways of IFN types I, II, and IL-6 through their respective receptors. Sequence of the ISRE, GAS, and pIRE response elements to which the tyrosine phosphorylated STAT proteins bind are shown at right (for details see text Sections II and IIIA). (B) Scheme of the IFN-β and IFN-α receptor complexes with intracellular tyrosine (Y) residues which, after phosphorylation by Jak1, Tyk2 tyrosine kinases, may be attachment sites for SH2-containing STAT factors. There are additional protein binding to the IC domains of IFNAR1, among them Phosphotyrosine phosphatase, Erk2- MAP kinase (David et al., 1995), and protein methyltransferase (Abramovich et al., 1996). The IFN-β receptor complex shows βPTyr, which may be IFNAR-2c linked differently than in the IFN-α complex (Platanias et al., 1996). There may be additional chains stabilizing the complex.

to form the ISGF3 transcription complex which controls the transcriptional induction of the various IFN-responsive genes (Fu et al., 1992; Pellegrini and Schindler, 1993; Darnell et al., 1994). ISGF3 binds to Interferon response sequences ISRE elements, found in the classical type I IFN-response genes such as 2'-5'oligo- A synthetase, MHC class I, PI/eIF-2 protein kinase, Mx genes, and others which in turn mediate the biological effects of IFN-α,β (Revel and Chebath, 1986; Benech et al., 1987). For example, antiviral effects on picornaviruses and cell antiproliferative effects can be mimicked by cDNA transfection of ds RNA activatable 2'-5' A synthetase (Chebath et al., 1987; Rysiecki et al., 1989; Chebath and Revel, 1992) or PI protein kinase (Meurs et al., 1992; Chong et al.,1992); the Mx protein, a GTPase in man, inhibits influenza virus specifically in mouse (Horisberger, 1992).

Stat1 also forms homo- or heterodimers which activate GAS/pIRE DNA elements in a different class of IFN-responsive genes induced by IFN-γ but also IFN-β (Shuai et al., 1994; Harroch et al., 1994a, 1994b), among which the gene for the IRF-1/ISGF2 transcription factor. Other Stat factors, in particular Stat3/APRF, are activated by IFN-β and bind to pIRE sequences of the IRF-1 gene (Harroch et al., 1994b). IRF-1/ISGF2 itself contributes some IFN effects by acting on ISRE sequences with IFN-induced ISGF3 or without it (Reis et al., 1992; Pine, 1992) and IRF-1 contributes to endogenous 2'-5' A synthetase level (Ruffner et al., 1993). The specificity of the Stat1-containing ISGF3 for ISRE depends on the DNA-binding ISGF3γ subunit, product of another gene of the IRF-1 family (Veals et al.,1992) and on the association of Stat2 with Stat1 which is specific to IFN-α,β (Improta et al.,1994). Interestingly, Stat2 (but not Stat1) was found associated along with Tyk2 on the IFNAR1 receptor chain (Abramovich et al., 1994b). It is likely that it is the interaction between the IFNAR1,2, and maybe other proteins, which brings in contact the Jak1, Tyk2, and Stat proteins which participate in the IFN-α,β signal transduction. These pathways, illustrated in Figure 3, form the framework in which differences between the actions of IFN-β and IFN-α can be discussed.

III. DIFFERENTIAL ACTIVITIES OF IFN-β

A. Differential Receptor Signaling

The specific involvement of the βPTyr receptor-associated component in the action of IFN-β described in the previous section indicates

that there may be some difference in the signal transduction and/or transcriptional factor activation pattern between IFN-β and IFN-α through their shared receptor system (Figure 3b). Further evidence along this line comes from studies of Tyk2-defective mutant cells, such as U1, selected for resistance to IFN-α but which still responds to a significant extent to IFN-β (Velazquez et al., 1992; Watling et al., 1992). The ability of Tyk2- mutant cells to retain some binding and response to IFN-β suggests that there may be a Tyk2 independent pathway available to IFN-β but not to IFN-αs (Uze et al., 1995). The Tyk2 protein has a tyrosine kinase TK domain in its C-terminal part and a TK-like (KL) domain in the middle part of the protein.Transfection of Tyk2- mutants by Tyk2 molecules lacking the TK domain (ΔTK) partially restores IFN-α binding and response, but IFN-β binding remains the same while response is increased (Velazquez et al., 1995). Therefore, Tyk2 by its KL domain contributes to the receptor binding of IFN-αs, probably through availability of functional IFNAR1, but not of IFN-β which may have alternative binding sites. The functional TK domain of Tyk2 is more important for certain gene response to IFN-α than to IFN-β, so that there may be a Tyk2-independent pathway for IFN-β in addition to the Tyk2-mediated common pathway used by IFN-α and IFN-β (Uze et al., 1995). The IFN-β-specific signal transduction may operate through alternative phosphorylations specific to IFN-β which bypass the Tyk2 defect. Possibly, a part of such an IFN-β specific binding/signaling pathway may be due to the stable linking of IFNAR2-2 to IFNAR1 (possibly involving other proteins), giving rise to the βPTyr band associated with IFNAR1 in response to IFN-β but not IFN-α (Abramovich et al., 1994b; Platanias et al., 1994, 1996). Recently, an ISRE-type gene induced by IFN-β (and IFN-γ) but poorly by IFN-α was found, however, for this β-R1 gene the Tyk2 kinase appears essential (Rani et al., 1996).

The operation of receptor-directed discrimination between IFN-β and certain IFN-α subtypes is also shown by transfection of the human IFNAR1 chain into rodent cells and measure of 2'-5' A synthetase induction: In mouse cells, response to human IFN-α8 but not to IFN-β is observed, whereas in hamster cells IFNAR1 mediates a response to human IFN-β but not to IFN-α8 (Abramovich et al., 1994a). This discrimination is probably due to the interaction of the human IFNAR1 chain with the other components of the Receptor contributed by the rodent cell, leading to hybrid receptor complexes which can better recognize one or the other IFN subtypes. This probably also explains other differences in species-specificity: bovine or porcine cells respond-

ing to IFN-α but not to IFN-β, the latter being in contrast more active than IFN-α on rabbit cells (Hayes, 1981).

B. Cell Growth Inhibition and Gene Expression

That similar discrimination between IFN-β and IFN-α does operate in human cells is shown by a number of cell lines isolated from human tissues. Brain tumor-derived glioma cells are growth-inhibited by IFN-β but very little by IFN-α2, although the binding of both subtypes is similar on these cells (Rosenblum et al.,1990). IFN-β induces in such cells a 10 times higher induction in the expression of genes such as 2'-5' A synthetase (2'-5'AS) or 68 kDa PI/eIF-2 protein kinase than does IFN-α2, indicating a difference in intracellular signaling. Growth inhibition is related to these gene inductions (Section II) and probably involves several oncogene-suppression mechanisms (Lengyel, 1993). Higher sensitivity to the antiproliferative effect of IFN-β than of IFN-α 2 or a4 is also seen in human melanoma cell lines, but their binding affinity for IFN-β is also higher than for IFN-α (Johns et al., 1992). Similarly, vascular smooth muscle cells were reported to be more sensitive to growth inhibition by IFN-β than IFN-α (Palmer and Libby, 1992), which may contribute to a higher anti-angiogenic effect of IFN-β, so important for anti-tumor action (Sidky and Borden, 1987). A study of 25 cancer cell lines showed that 15 were more growth-inhibited by IFN-β, nine were inhibited by both IFN-α and IFN-β, and one (Daudi) more growth inhibited by IFN-α (Borden et al., 1982). Interestingly, IFN-α inhibits the growth of human erythroid progenitor cells (BFU-E) whereas IFN-β does not (Michaelevicz and Revel, 1987; Michalevicz et al., 1988). The last study also indicates that IFN-β is active on hematopoietic cells of hairy cell leukemia which have acquired resistance to IFN-α2.

A potentially important differential activity of IFN-β is the inhibition of gelatinase (type IV collagenase) production by cancer cells. Gohji et al. (1994) reported that human (CHO-made) recombinant IFN-β, but not IFN-α, suppressed gene transcription and production of the 72 kDa gelatinase in KG2 renal carcinoma cells as well as the invasion potential of the cancer cells which depends on such proteases attacking the extracellular matrix. This inhibitory activity of IFN-β, seen also with IFN-γ, was observed in the tumor cells but not in normal fibroblasts and may be a mechanism inhibiting metastatic growth in skin where fibroblasts can produce IFN-β (Fabra et al., 1992). The mechanism by which IFN-β inhibits expression of the deregulated

collagenase gene in metastatic cancer cells contrasts with its effect in normal skin fibroblasts where IFN-β (or α) stimulates collagenase and other metalloprotease genes through induction of the fos/jun AP1 transcription factor (Sciavolino et al.,1994). Reduction of protease secretion by cancer cells and the more potent and general growth-inhibitory function, are mechanisms characterizing direct actions of IFN-β on tumor cells. Another effect is the sensitization of lung cancer cells to the cytotoxic effect of gradiations, an effect not produced by IFN-α (Gould et al.,1984; see Section VIIIA). The increase in steroid receptors in hormone-dependent cancer cells, which is discussed in section VIIIA, may be an example of a tumor cell differentiation effect of IFN-β (see Section IVH).

C. Antiviral Effects

The mechanisms by which IFN-β inhibits the intracellular replication of numerous types of viruses are not different from those of IFN-α and have been reviewed (Revel, 1984; Chebath and Revel, 1986; Pestka et al., 1987; Sen and Lengyel, 1992). Inhibition of viral mRNA translation is a general mechanism which is explained by induction of the 2'-5' A synthetase/RNase L system and the PI/eIF-2 protein kinase, both activated by viral ds RNA and both mimicking IFN effects on picornaviruses when expressed by gene transfection (Chebath et al., 1987; Meurs et al., 1992). Other actions on viral transcription or RNA replication are probably also mediated by IFN-induced proteins, an example being the Mx proteins inhibiting influenza replication specifically in mouse cells (Horisberger, 1992), but the functions of many of the induced proteins is still unclear. It is likely that different induced proteins act on different viruses, some probably also causing membranal changes which impair assembly of certain viruses, among which retroviruses and VSV, or impair virus uncoating. The antiviral mechanisms are under complex regulation, a number of viruses having genes which inhibit IFN effects (Stark and Kerr, 1992; Sen and Lengyel, 1992). It is, therefore, not surprising that IFN-β exhibits certain differences with IFN-α in its anti-viral action. For example, IFN-β triggers faster development of the anti-viral state against VSV in skin diploid fibroblasts and different dose-response slopes as compared to IFN-α (Hayes, 1981). Therefore, *in vivo*, the anti-viral activity of IFN-β will depend on the cell and tissue-specific characteristics of IFN-β action, but also on several immune-mediated effects on infected cells.

D. Effects through the Immune System

An important activity of both IFN-α and β is the up-regulation of histocompatibility class I antigens (MHC-I, HLA-A,B,C) which present peptide antigens to $CD8^+$ cytotoxic lymphocytes(CTL) or suppressors (see Rosa et al., 1986 for review). In several virus-infected cells, it was observed that IFN-β is actually the mediator of MHC-I induction since anti-IFN-β, but not anti-IFN-α, antibodies block the HLA-class I increase in human glioma and vascular endothelial cells infected by measles virus (Dhib-Jalbut and Cowan, 1993). The enhancement of these MHC surface antigens and others such as tumor-associated and carcinoembryonic antigens, and of adhesion molecules such as ICAM-1 explain some of the immuno-mediated actions of IFN-α and β against tumor cells and pathogens (Borden, 1988; Giacomini et al., 1990; Kraus et al., 1992; Sansonno et al., 1992). Other cytokines, IFN-γ or TNF, have stronger effects on surface antigens and strongly induce MHC class II molecules, sometimes leading to auto-immune reactions: in this cases, IFN-β acts as inhibitor of MHC-II induction and as promoter of suppression (see Section VIIA). IFN-β itself may have some effects on MHC-II expression *in vitro* (Dolei et al., 1982; Rosa et al., 1986) and *in vivo* administration of IFN-βser enhances HLA-DR,DQ on monocytes although transiently as compared to the effect on HLA-A,B,C (Goldstein et al., 1989; Borden et al., 1990).

Among indirect effects mediated by immune cells which lead to tumoricidal and microbicidal actions of IFNs, the production of nitric oxide (NO) by monocyte/macrophages requires specifically IFN-β. Thus, the induction of NO synthase mRNA and protein and production of NO by lipopolysaccarides (LPS) in macrophages is blocked by anti-IFN-β antibodies specifically and exogenous IFN-β provides an essential signal (possibly IRF-1) for NO production in response to bacterial stimuli (Zhang et al., 1994; Fujihara et al., 1994). Induction of indoleamine 2,3-dioxygenase (IDO) in macrophages appears related to their ability to generate reactive oxygen radicals for tumoricidal-antimicrobial actions: IDO is induced by the macrophage activator IFN-γ, but IFN-β is independently capable of inducing IDO and potentiates the effect of LPS (Carlin et al., 1989). Superoxide production by neutrophils is also enhanced by IFN-β (Brunelleschi et al., 1988). On the lymphocyte effector arm, type I IFNs mainly activate natural killer (NK) non-MHC restricted cells and this is seen *in vitro* and *in vivo* in response to natural or recombinant IFN-β (Lucero et al., 1981; Goldstein et al., 1989; Borden

et al., 1990; Liberati et al.,1991). In cancer patients, the depressed cytotoxic activity of white blood cells can be enhanced by IFN-β which increases $CD16^{+}$ $CD3^{-}$ NK cells (Molto et al., 1994), the effect of IFN-β being synergistic with that of IL-2 (Liberati et al., 1994a). NK enhancement may correlate with the *in vivo* tumor response to IFN-β, and also contribute to the anti-viral effect on HTLV-I (Fuggetta et al., 1990). IFN-β also increases cytotoxicity of T-lymphocytes, this varies according to the donor but always in correlation with the induction of typical IFN markers such as 2'-5' oligo A synthetase (Gazitt et al.,1984). In addition, IFN-β was reported to also increase the sensitivity of target endometrial tumor cells to cell-mediated cytotoxicity (Rossiello et al., 1994). Some of these immuno-mediated effects of type I IFNs must be essential for their *in vivo* anti-tumoral action since the latter is abolished in T cell-depleted mice (Gresser et al., 1990).

IV. HOW, WHEN, AND WHERE IS IFN-β PRODUCED?: IFN-β GENE EXPRESSION

The distinctive behavior and activities of IFN-β probably stems from the specific modalities of its production in the human body, which are quite different from the IFN-α subtypes, as reviewed in this section.

A. Gene Locus

Human IFN-β is encoded by a gene on chromosome 9 bands p22-p21, which is telomeric to the large cluster of 25 IFN-α,ω genes whose map structure has been established as a YAC clones contig of about 400 kb (Diaz et al., 1994). The IFN-β gene (IFNB1) is about 50 kb distal from the first gene of this cluster, IFNW1, and the structure of a 36 kb DNA region around the IFN-β gene is known (Gross et al., 1981).The IFN-β-transcribed gene is 777 bp long (900 with poly A tail); the gene is intronless and can be expressed into active IFN-β in bacteria (Mory et al., 1981). The homology of the IFN-β DNA sequence to the group of IFN-α genes is around 50-60% (vs. up to 90% homology in the IFN-α genes), the separation of β from α genes probably having occured by duplication very early in evolution of this genomic region, maybe 200 million years ago (Weissmann and Weber, 1986). In bovine, ovine, and porcine DNA, several IFN-β genes are found, but in murine and human DNA the IFN-β1 gene is unique.

Deletions in the IFN gene cluster occur in certain cancer cells (Diaz et al.,1988, 1994). Glioma cells, non-small cell lung carcinoma and T-Acute lymphocytic leukemia cells, are often found to have homozygous deletions encompassing the IFN gene locus on chromosome 9p21-22 (Coleman et al., 1994; James et al., 1993; Schmidt et al., 1994). The loss of the IFN-β gene might be associated with late stage, highly malignant glioblastomas grade II and IV (Sugawa et al., 1993). However, the significance of such deletions analyzed in various tumors may actually relate to a tumor suppressor gene located between the IFN locus and the more centromeric D9S171 marker (Cheng et al., 1993). Melanomas have chromosome 9p21-22 deletions which are distinct from those of gliomas (Coleman et al., 1994).

B. Induction and Superinduction

Expression of the IFN genes and secretion of IFN from cells is essentially an inducible process which can be triggered by infection with a number of viruses and for which double stranded (ds) RNA is considered to be the "proximal inducer" (see Marcus, 1983,1987 for review). Live viruses which are cytopathic are not as effective IFN inducers as inactivated viruses or dsRNA. The mechanism of action of ds RNA is still a challenging question, one molecule per cell being sometimes able to induce IFN, suggesting that it acts through activation of enzymes such as the ds RNA dependent protein kinase (Kumar et al., 1994) or possibly proteases (Palombella and Maniatis, 1992). Many chemical inducers of IFN have been described in addition to various synthetic ds RNA derived from poly rI:rC (see Levi and Salazar, 1992 for review). Poly rI:rC is a preferential inducer of IFN-β as compared to IFN-α (see Sections IVF and IV), but viruses and also bacteria can induce IFN-β in fibroblasts (VanDamme et al.,1989). It is unlikely that all these inducers act in the same way or have a single action. DsRNA is an inducer of many proteins (Raj and Pitha, 1980). IFN induction is a complex process involving transcriptional activators and repressors as well as post-transcriptional controls, the inducers probably acting on all these processes.

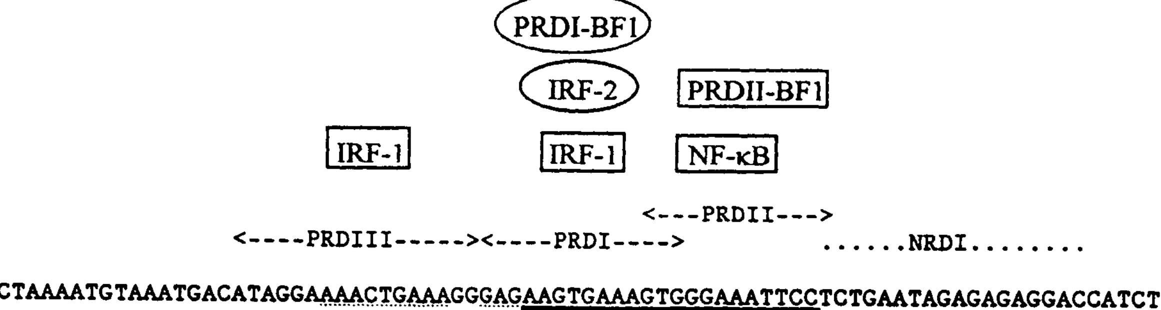
Transcriptional activity of the IFN-β gene in non-induced cells is very low or undetectable, and is clearly switched-on in response to inducers such as poly rI:rC, as shown with nuclei from induced fibroblasts (Raj and Pitha, 1983; Nir et al., 1984). Accumulation of IFN-β transcripts is observed from 1-1.5 hours to 6-12 h depending on the cells but the levels of IFN-β mRNA decrease again rapidly due to RNA degradation (Cavallieri et al., 1977; Sehgal et al., 1977; Raj and Pitha, 1983) and to

transcriptional repression (Whittemore and Maniatis, 1990). The induction of IFN-β gene transcription and the accumulation of IFN-β mRNA do not require protein synthesis in most cell types (Sehgal et al., 1979; Nir et al., 1984; Whittemore and Maniatis 1990). The post-induction decrease in IFN-β mRNA depends on protein synthesis and is inhibited by cycloheximide (CHX) leading to the superinduction phenomenon seen when the poly rI:rC stimulus is given with cycloheximide (and better if followed at 4 h by Actinomycin D), superinduction being a combination of increased stability of IFN-β mRNA (Cavallieri et al., 1977; Sehgal et al., 1979; Raj and Pitha, 1983) and of reduced transcriptional repression (Whittemore and Maniatis, 1990; Palombella and Maniatis, 1992). Priming by IFN prior to the poly rI:rC stimulus is another way to increase IFN-β production (Stewart, 1979); priming requires protein synthesis (Fujita and Kohno, 1981), enhances transcription of the IFN-β gene (Nir et al., 1984) and formation of transcripts extending beyond the normal polyadenylation site (Content et al., 1983). Thus, induction of IFN-β should be discussed both in terms of transcriptional controls of the IFN-β gene and of post-transcriptional controls which eventually determine IFN-β mRNA accumulation in the cytoplasm.

C. Transcriptional Control Elements of the IFN-β Gene

The 5' flanking region of the IFN-β gene contains regulatory sequences which are necessary for virus and dsRNA inducibility and confer inducibility to reporter genes (Taniguchi, 1989; for review of the early evidence see Revel, 1983). Deletions and mutation analyses in different cell systems were at first hard to reconcile. Presently, four adjacent and sometimes overlapping regulatory sites have been characterized from position -105 to -55 respective to the cap site (Figure 4). The observation that the hexamer AAGTGA at -76/-71 and certain variants of this motif in repeated tandem copies could mediate virus-inducible transcription (Fujita et al., 1987) helped define a site to which binds a 48 Kda Interferon-Regulatory Factor, IRF-1 (Miyamoto et al., 1988). IRF-1 can act as a positive transcription factor but its activity is repressed by negative factors, among them IRF-2 which has a homologous N-terminal DNA binding domain and the same binding specifity (Harada et al., 1989,1990; Tanaka et al., 1993). The binding sequences and functions of IRF-1 are further discussed following. This first site or Positive Regulatory Domain I, (PRDI, -79/-66; Figure 4) was also defined by deletion analyses and distinguished from the adjacent induc-

REGULATORY REGIONS IN THE IFN-β GENE PROMOTER



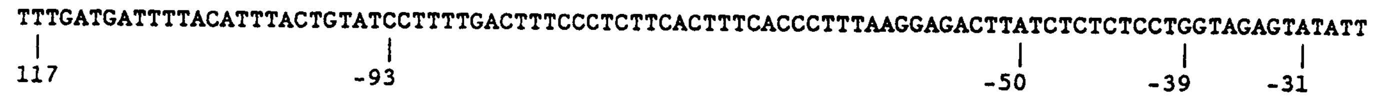


Figure 4. Promoter region of the human IFN-β gene with sequence of regulatory elements and binding proteins. Nucleotides involved in protein binding are underlined (for details see text Section IV)

ible PRDII site (-68/-57; Goodburn et al., 1985; Goodburn and Maniatis, 1988). PRDII is a binding site for NF-κB and comparable factors such as H2TF1, which are known transcription activating factors of Ig and MHC-I genes and which through PRDII multimers also mediate IFN-β induction by virus and ds RNA (Fujita et al.,1989b; Lenardo et al.,1989; Visvanathan and Goodburn, 1989; Hiscott et al., 1989). The third site PRDIII (-96/-80) is a virus-inducible element when multimerized (Leblanc et al.,1990) and to which IRF-1 binds but with much lower affinity than to PRDI (Fujita et al., 1988). This partially supports the notion of multiple IRF binding sites (hexamer motifs) in the -117/-65 region (Taniguchi, 1989). Cooperation in the function of the PRDI,III/ IRF and PRD-II/ NF-κB sites is an essential feature of the IFN-β gene regulation (Dinter and Hauser, 1987; Fan and Maniatis, 1989; Leblanc et al., 1990; Garoufalis et al., 1994) and these sites have both positive and negative regulatory proteins (see following). However, a fourth site PRDIV was identified at -101/-91 as a binding site for a member of the ATF/CREB (Activating Transcription Factor cAMP Response Element Binding) protein family (Du and Maniatis, 1992). PRDIV mutations decrease virus induction and multimers can confer virus and cAMP inducibility in some cells or constitutive expression in others. In addition, a negative regulatory domain called NRDI (-56/-39) may function between PRDII and theTATA box at -31 (Goodburn et al., 1986).

D. Function of Proteins Binding to the IFN-β Transcriptional Elements

Despite the evident combinatorial nature of the IFN-β gene regulatory elements, it is simpler to consider the type and functions of proteins binding each element in the induction and in the repression of the gene transcription.

PRDII, PRDIV: Roles of NF-κB, HMG-I, ATF/CREB

On PRDII, binding of the NF-κB subunits p65 and p50 or c-Rel occurs in response to Sendai virus infection before IFN-β transcription starts; the composition of the complex may vary with time, with possibly some functional implication (Garoufalis et al., 1994). A virus-induced degradation of the I-kappa B alpha (MAD3) inhibitor 2-8 h after infection appears to be the trigger of NF-κB activation, and a later resynthesis of this inhibitor may be related to the subsequent down regulation of the IFN-β gene (Garoufalis et al., 1994). NF-κB binding is activated by poly

rI:rC (Visvanathan and Goodburn, 1989) but also by cycloheximide (Sen and Baltimore, 1986). In addition to NF-κB which interacts in the DNA major groove of PRDII, the High Mobility Group protein HMG-I (Y) interacts in the minor groove (Thanos and Maniatis, 1992). Mutations of PRDII show that virus induction requires binding of both HMG-I (Y) and of the NF-κB p50-p65 heterodimer as is also indicated by antibodies (Thanos and Maniatis, 1995). HMG-I (Y) appears as a key architectural component in the assembly of the inducible transcription activation complex for both NF-κB on PRDII and for ATF-2/CREBP on PRDIV (Du et al., 1993). The combination of sites for NF-κB and ATF-2/CREBP with HMG-I seems related to TNF inducibility in genes such as E-selectin (Whitley et al., 1994), the IFN-β gene being also TNF inducible (Fujita et al., 1989c) but having additional control regions such as the IRF sites. With the entire IFN-β gene promoter, NF-κB and IRF-1 coexpression has a synergistic effect on induction demonstrating the specific combinatorial activity of this promoter, which is further emphasized by the fact that overexpression of either the I-kappa B inhibitor or IRF-2 repressor abrogates the induction (Garoufalis et al., 1994). The single PRDII element in the IFN-β gene does not work alone but needs to be either repeated or in a tandem of PRDI-PRDII single copies to confer dsRNA and virus inducibility (Fan and Maniatis, 1989; Leblanc et al., 1990). An additional protein binding to PRDII is PRDII-BF1 (about 300 kDa) whose role is unclear (Fan and Maniatis, 1990).

PRDI, PRDIII: The Role of IRF-1 and Other Proteins

PRDI is the site of action of multiple proteins with positive or negative transcriptional effects. A single copy of PRDI placed near a viral enhancer confers virus inducibility (Naf et al., 1991) and may act as a silencer in the absence of induction (Fujita et al., 1988). Expression of the IRF-1 transcription factor can induce constructs with tetramers of the sequences GAAAG(T/C) or GAAAC(T/C) and less GAAAGG, such sequences (as dimers) being found in PRDI and PRDIII of the IFN-β gene; instead, GAAATG found in the IFN-α promoters did not respond to IRF-1 (MacDonald et al., 1990). Tetramers of GAAAG(T/C) and GAAAC(T/C) had also a silencing effect, whereas GAAACG tetramers gave constitutive expression. The role of IRF-1 as positive transcription factor on multimers of PRDI sequences or on the IFN-β promoter is, however, a complex question. In mouse embryo carcinoma P19 cells which lack IRF-1 and IRF-2, expression of IRF-1 cDNA activates the

IFN-β promoter of endogenous and reporter genes, whereas IRF-2 represses IRF-1 (Harada et al., 1990). In other cells, such repressions may explain why IRF-1 has only low effects on the IFN-β promoter (Fujita et al., 1989a; McDonald et al., 1993) Reducing IRF-1 by antisense cDNA strongly inhibited IFN-β induction by virus and ds RNA, induction being restored by IRF-1 (Reis et al., 1992). However, in other systems no such correlation was found (Pine et al., 1990). Experiments with fibroblasts derived from IRF-1$^{0/0}$ knock-out mice show an IRF-1 requirement for IFN-β induction by poly rI:rC but not for induction by virus such as NDV (Matsuyama et al., 1993). IRF-1$^{0/0}$ embryo carcinoma stem cells also show IFN-β and α induction by NDV (Ruffner et al., 1993). An important observation is that priming of IRF-1$^{0/0}$ cells by IFN restores induction by poly rI:rC suggesting that priming induces another pathway which does not require IRF-1 and may be the same as the one used by virus inducers (Matsuyama et al., 1993). An IFN-induced factor needed for IFN-β induction had been proposed (Enoch et al., 1986) and may replace IRF-1 which itself is IFN-inducible (Harada et al., 1989). Hence, two questions should be discussed: the mode of action of IRF-1 in dsRNA induction and the other factor(s) which may replace it on the PRDI or III domains.

The functional activation of IRF-1 seems to be controlled both by induced synthesis of the protein and by post-translational activation. The promoter of the IRF-1 gene (Sims et al., 1993) contains a palindromic Interferon Response Enhancer (pIRE) which is a target for the Stat1 (p91) transcription factor activated by tyrosine phosphorylation in response to IFN-γ (Kanno et al., 1993) and for Stat3 or Stat1-Stat3 heterodimers activated by Interleukin-6 and IFN-β (Harroch et al., 1994b). Hence, type I IFNs and in particular IFN-β activate both the Stat1,3 proteins which can induce IRF-1, but also Stat2 which with Stat1 is part of the ISGF3 transcription complexes activating IFN-response genes (Darnell et al., 1994; see previously in Section II). Accumulation of IRF-1 is induced by viruses, ds RNA (Miyamoto et al., 1988) and by Stat-mediated cytokines IFN-γ, IL-6, and IFN-α,β (Harada et al., 1989; Pine et al., 1990; Harroch et al., 1994a) but also by Stat-independent IL-1 or TNF (Fujita et al., 1989c). However, in line with the induction of IFN-β transcripts without protein synthesis (Section IVB), IRF-1 also appears to be activated post-translationally by a process involving protein phosphorylation and which is triggered by dsRNA and viruses but not by TNF or IFN-β (Watanabe et al., 1991). This activation can explain the strong IFN-β induction by dsRNA, and weak induction by TNF although both

induce IRF-1 synthesis and activate NF-κB (Fujita et al., 1989c). By such activation, IRF-1 could mediate IFN-β gene induction by dsRNA, but this may not be its main function. IRF-1 has other important activities such as growth inhibition and antioncogenic effect (Kirchoff et al., 1993; Harada et al.,1993). IRF-1 seems able to enhance expression of IFN-responsive genes by some cooperative effect with ISGF3 on ISRE enhancers resembling PRDI (Harada et al., 1990; Reis et al., 1992; Pine, 1992) although IRF-1 is not required for some IFN actions (Matsuyama et al., 1993). Interestingly, IRF-1$^{0/0}$ mice show a role for IRF-1 in CD8+ T-cell development, in apoptosis and in NO synthase induction (Matsuyama et al., 1993;Tanaka et al., 1994).

Other proteins interacting with IRF-1 sites.The other proteins known to bind PRDI motifs seem to be transcriptional inhibitors. IRF-2 antagonizes IRF-1 actions including those on growth-inhibition since IRF-2 has oncogenic effects (Harada et al., 1993). The IRF-2 gene promoter itself has an IRF-1 binding site strengthening the notion of a balanced IRF-2 /IRF-1 regulation (Harada et al., 1994). Cells from IRF-2$^{0/0}$ mice show a two to threefold higher IFN-β mRNA peak after NDV induction, suggesting that IRF-2 represses the IFN-β gene (Matsuyama et al., 1993). However, this was not seen with dsRNA and the post-induction decrease of IFN-β mRNA occurred in the IRF-2 deficient cells; the lack of IRF-2 did not cause constitutive expression either. Another repressor of the IFN-β gene, blocking its viral induction, is the 88 kDa PRDI-Binding Factor-1, a protein with five zinc fingers unrelated to IRF-1 and which may play a role in the pre-or post-induction shut-off (Keller and Maniatis, 1991,1992). Other proteins binding PRDI appear IRF related. A factor from myeloid cells, interferon consensus sequence binding protein (ICSBP) has an N-terminal DNA-binding domain homologous to IRF-1 and 2, and acts as transcriptional repressor on PRDI (Weisz et al., 1992).

A factor, PRDI-BFi, whose binding to PRDI is induced by virus or poly rI:rC even in the presence of cycloheximide (CHX), turned out to be a form of IRF-2 with a C-terminal truncation which reduces its ability to inhibit IRF-1 transcriptional activity (Palombella and Maniatis, 1992). This factor is probably similar to TH3 (Cohen and Hiscott, 1992). The IRF-2 present constitutively (PRDI-BFc), undergoes an induced proteolytic truncation which is induced 2 h after CHX alone and 8 h after virus infection and could play some role in derepression of the IFN-β gene. Although CHX leads to proteolytic activation of both NF-kB and PRDI-BFi, it is not sufficient to induce the IFN-β promoter (Palombella and Maniatis, 1992). Still, CHX enhances the level of IFN-β gene

induction (Pine et al., 1990) and the effects of CHX on transcription were seen in some IFN-β gene constructs (Ringold et al., 1984). Deleted forms of the IFN-β gene (with only the NRDI region) in viral episomes have shown CHX induction but this is not likely to be due to IRF-2 processing (Nir et al., 1984). Since CHX delays the post-induction shut-off of the gene, which partly explains superinduction (Whittemore and Maniatis, 1990), the derepression produced by IRF-2 truncation may prolong expression late after induction. Shut-off does occur in IRF-2°/° cells indicating that additional shut-off mechanisms operate but, nevertheless, loss of IRF-2 leads to an enhanced induction level (Matsuyama et al., 1993). Disappearance of constitutive IRF-2 (and ICSBP) factors with truncation of IRF-2 was observed after IL-6 induction of myeloid M1 cells, and in this case IRF-2 phosphorylation was seen to modulate its processing (Harroch et al., 1993; Ben-Simchon et al., 1995). Removal of such repressors, binding to PRDI/IRF and ISRE elements, could influence the response of various genes to a number of inducers.

How Does Induction of IFN-β Transcription Work?

To summarize, a number of positive and negative transcriptional factors bind the different domains of the IFN-β gene promoter. In the induction by dsRNA, the synergism resulting from activation of IRF-1 plus NF-κB and their binding to PRDI and PRDII probably plays the major role. In virus-dependent induction, IRF-1 appears dispensable and another positive transcription factor may replace IRF-1. This other factor may not operate in all cells since some do require IRF-1 (Reis et al., 1992). It is safe to assume that at least two independent mechanisms of induction exist in different cells, one of which is sensitive to 2-amino purine, a protein kinase inhibitor (Daigneault et al., 1992). There is evidence that induction of the IFN-β gene by dsRNA and by virus involves some phosphorylation and can be inhibited by 2-amino purine (Zinn et al., 1988) or staurosporin (Watanabe et al., 1991) but we do not know how dsRNA and viruses really work. Viruses may change more cell components than does dsRNA, such as cAMP levels influencing PRDIV. DsRNA may trigger some enzymes, such as the IFN-inducible protein kinase PI (PKR) which by phosphorylating I kappa B activates NF-κB: PKR°/° mice show reduced IFN induction by dsRNA but the PKR absence is replaced by priming with IFN before dsRNA, and PKR is not required for viral inducers (Yang et al., 1995). It is unlikely to be simple. Poly rI:rC directly activates IFN-inducible genes by an unknown

mechanism different from the IFN-dependent activation of ISGF3 (Decker, 1992). Hydrolysis of phosphatidyl-inositol is required for poly rI:rC and virus induction of IFN-β, suggesting a role for these second messengers (Lin et al., 1991). Clearly, many questions must still be answered to elucidate the action of the numerous inducing agents (virus, dsRNA, cAMP, cytokines) acting on the IFN-β gene transcription factors.

E. Post-transcriptional Controls

Stabilization of the IFN-β transcripts plays an important role in the phenomenon of superinduction, which has been instrumental in allowing the production of large amounts of IFN-β from human diploid fibroblasts cultures (Sehgal and Tamm, 1979). Typically superinduction, achieved by combining poly rI:rC and CHX for several hours and then replacing these by Actinomycin D, increases the level and stability of IFN-β mRNA and the amount of IFN-β secreted from the cells. In fact, nuclei continue to synthesize IFN-β transcripts for many hours after induction, but (without superinduction) these transcripts do not accumulate at late times in the cells and IFN secretion stops (Raj and Pittia, 1983). Further evidence for post-transcriptional control comes from experiments with IFN-β gene constructs deleted of all sequences upstream of the NRDI and TATA box regions and placed in an episomal Bovine papilloma virus vector. In this case, constitutive nuclear transcription—without mRNA accumulation—was observed, and poly rI:rC alone or CHX alone both induced the accumulation of IFN-β mRNA indicating that poly rI:rC itself may have a post-transcriptional effect (Nir et al., 1984).

F. Differences between IFN-β and IFN-α Gene Controls

It is important to realize that despite the apparent sequence homologies between the promoters of the IFN-β and of the IFN-α genes, the transcriptional controls are quite different. The IFN-α1 gene promoter lacks a NF-κB binding site and this factor, crucial for IFN-β, does not seem involved in the IFN-α promoter (Lenardo et al.,1989; MacDonald et al., 1990). The virus response element VREα1 has a sequence called TG domain, which like the GAAATG tetramer, does not respond to IRF-1 although it mediates virus inducibility when attached to an enhancer (MacDonald et al., 1990; Naf et al., 1991). Protein binding to the VREα element are not competed by IFN-β promoter elements (Hiscott et al., 1989). IRF-1 binds the IFN-α promoter one order of magnitude

less than it binds the IFN-β promoter (maybe due to PRDIII-like sequences) which may explain some IFN-α induction in cells overexpressing IRF-1 (Fujita et al., 1989a; Au et al., 1992). A TG factor which binds VREα—but not the corresponding sequences of the IFN-β promoter is a candidate for the induction of IFN-α genes, whereas Oct-1 type factors - binding a octamer sequence of an immunoglobulin gene which has homology to VREα1—give constitutive activity (MacDonald et al., 1990). In undifferentiated embryonic stem cells IFN-α induction by viruses was higher than in differentiated cells, whereas IFN-β induction was exclusively a property of the differentiated fibroblast-like cells (Ruffner et al., 1993). Study of IRF-$1^{0}/^{0}$ cells showed that IRF-1 does not play a role in the virus induction of the IFN-α gene, but can affect somewhat the level of IFN-β gene induction (Ruffner et al., 1993). However, with poly rI:rC, IRF-$1^{0}/^{0}$ embryo fibroblasts have a defect in both IFN-α and IFN-β mRNA induction (Matsuyama et al., 1993). The general conclusion is that differences in the transcriptional domains of the IFN-α and IFN-β gene promoters and their regulatory proteins predict that these two subtypes of IFN will be induced differently in cells and tissues. The fact that IFN-β has to be glycosylated during the secretion process through the endoplasmic reticulum, which is probably not the case for many IFN-α subtypes (Pestka et al., 1987), further differentiates the IFN-β biosynthetic process.

G. Expression of IFN-β versus IFN-αs in Cells and Tissues

In general, IFN-β is the main subtype made by fibroblasts and epithelial cells, that is, cells from solid tissues, whereas IFN-αs are more efficiently induced in blood leukocytes. This is not an absolute division, and the amount of each subtype produced depends on the inducer as well as on the cell type. When induced by ds RNA such as poly rI:rC, human diploid fibroblasts exclusively produce IFN-β, but can also produce variable amounts of IFN-α when viruses such as the Newcastle disease virus are used as inducers (Hayes et al., 1979). In leukocyte buffy coats which are virus induced, IFN-β represents about 1% of the IFN produced; in lymphoblastoid cells, 10-20% is IFN-β (Hayes, 1981). Each individual IFN-α gene is also variably induced in different blood cell types (Weissmann and Weber, 1986). Most, if not all, human cell strains that have been tested respond to poly rI:rC induction and superinduction, although the amount of IFN produced varies (Meager et al., 1979). Human embryonic trophoblasts also produce IFN-β alone with poly rI:rC

as inducer, and IFN-β together with some IFN-αs andIFN-ω with viral inducers (Aboagye-Mathiesen et al., 1993). Poly rI:rC and viruses will induce other cytokines such as IL-6 (formerly IFN-β2) and GM-CSF along with IFN-β, indicating a common cytokine gene regulation (VanDamme et al., 1989). The same study showed that in human diploid fibroblasts, IFN-β was strongly induced by measles virus, less by rubella virus but very well by bacteria such as *E.coli*, similarly to the other cytokines.

IFN induction is most likely part of the cytokine response which triggers the defense of the organism during infections. The role of IFN-β with respect to the IFN-α subtypes and other cytokines could, therefore, be considered in the setting of an ongoing infection. In a murine model system using UV-inactivated herpes virus given intraperitoneally, the IFN activity detected in the peritoneal cavity within 2 h was all IFN-β, whereas the serum peak at 4-8 h was due to IFN-α. The only detectable source of IFN-α in these mice was bone marrow, where 25% was IFN-α and 75% was IFN-β, the IFN activity in blood mononuclear cells and spleen being also mainly IFN-β (Bhuiya et al., 1994). These findings would be compatible with a model in which IFN-β is made by most tissue cells early after infection but remains localized, whereas IFN-α made by "natural IFN-producing cells" which are HLA-DR and CD4 positive (Sandberg et al., 1990; Bhuiya et al., 1994,) is circulating in the blood. Although extrapolation to humans and other viruses is difficult, it is tempting to consider the respective roles of IFN-β and IFN-α in light of such results and in light of their pharmacodynamic differences (discussed in Section VI below). IFN-β may be viewed as the subtype which can be made by most tissues where the infection initiates, acting then locally to prevent spread of the infection through a very rapid induction of the anti-viral state (Hayes, 1981; Dianzani, 1992), and, thereafter, diffusing slowly through the lymphatic system (Bocci et al., 1988). In contrast, IFN-αs are made by certain hematopoietic cells and circulate in the blood stream as a less localized system of defense. This distinction between the origins and roles of IFN-β and IFN-α , illustrated in Figure 5, may be a useful concept when considering their respective clinical applications and mainly the differing intensities of their side effects (see Sections VI and VII).

H. Autocrine IFN-β

Evidence that IFN-β may even be a physiological product in the normal (non-pathological) cell life comes from the observation that

Figure 5. Physiological differences in the sites of production of IFN-β and IFN-αs during virus infections and subsequent diffusion, accounting for the pharmacokinetic difference between the two subtypes (see text Sections IVG and VIA).

IFN-β is spontaneously secreted by cells undergoing growth transitions and differentiation (see Revel et al., 1987 for review). In differentiating human monocytic U937 cells, the autocrine secretion of low amounts of IFN-β is responsible for the induction of MHC-I expression and the strong increase in 2'-5' A synthetase (Yarden et al., 1984). Murine erythroleukemic as well as myeloleukemic M1 cells produce low amounts of an IFN-β during differentiation and in the latter case, blocking IFN-β by antibodies partly prevented the growth arrest and c-myc reduction (Resnitzky et al., 1986). This may depend on the inducer of differentiation since when IL-6 is used as inducer the growth-arrest and phenotypic differentiation occur even with anti-IFN antibodies (Gothelf et al., 1991). IL-6 induces IRF-1 which itself contributes to growth inhibition (Abdollahi et al., 1991; Harroch et al., 1993) and to the small induction of IFN-β mRNA (Bickel et al., 1990). The autocrine IFN-β produced is too low to act by itself and in fact the strong induction of IFN-activatable genes such as 2'-5' A synthetase or MHC-I results

from a synergism between IL-6 and IFN-β (Cohen et al., 1991), IL-6 activating Stat1 and IRF-1, but IFN-β being essential for the required Stat2 activation (Harroch et al., 1994a). The role of autocrine IFN, probably IFN-β, in maintaining the MHC-I surface antigen expression in mouse fibrosarcomas, carcinomas, and melanoma cell lines was demonstrated, indicating that the immuno-reactivity of malignant cells may be critically dependent on their ability to spontaneously secrete IFN (Nanni et al., 1992). Endogenous IFN-β is critical for induction of nitric oxide production in macrophages (Fujihara et al., 1994). Spontaneously produced IFN-β seems involved in myogenesis (Birnbaum et al., 1990) and contributes to the fibroblastic differentiation of embryonal carcinoma P19 cells by retinoic acid, possibly by influencing exit from cell cycling (Belhumeur et al., 1993). Cell cycle related changes in 2'-5' A synthetase have led to findings of autocrine IFN-β production and its implication in Go and G1 transitions (Resnitzky et al., 1986; Wells and Mallucci, 1988). However, activation of the ISGF3 complex (Stat1/Stat2) and of IRF-1 mediating IFN effects are also seen during S/G2M transit, again as a result of autocrine IFN-β secretion which if blocked by antibodies markedly accelerates the transit of human myeloma cells to the next G1/S phase (Ben-Simchon, 1995). Taken together, these data strongly indicate a physiological function of autocrine IFN-β as a homeostatic regulation of cell cycling.

V. FORMS OF IFN-β AVAILABLE FOR CLINICAL USAGE

A. Natural Fibroblast IFN-β

The most common source is from cultures of human fibroblasts established from foreskin obtained at circumcision. Fibroblasts growing from individual explants can show very different levels of IFN-β production when subjected to the superinduction procedure with poly rI:rC, cycloheximide and actinomycin D (see Sections IVB and IVE), and the best producers are selected (titers around 20,000 IU/ml culture). Other criteria in the selection are strict diploidy of the fibroblasts, absence of any pathogen, number of passages possible until senescence, stability of production, and titers obtained in two harvests at 24 and 48 h post-superinduction. Once selected, a line derived from one foreskin can be used for years by growing batches of cells which are then superinduced, harvested and discarded. The basic methodology for natural IFN-β production and downstream processing (Leong and Horoszewicz, 1981;

Knight, 1981) has evolved as newer technologies for large-scale fibroblast cultures, in particular on microcarriers, have become available allowing industrial plants to produce yearly around 10 grams or 3 million megaunits of highly purified IFN-β for clinical uses.

B. Recombinant IFN-β from Mammalian Cells

A very efficient constitutive production of human IFN-β was developed in Chinese hamster ovary (CHO) cells by introducing the IFN-β gene coding sequence under the control of the Simian virus 40 early promoter with appropriate polyadenylation signals (Chernajovsky et al., 1984). Secretion of IFN-β without need for induction and at titers of 300,000 IU/ml (i.e., more than 10 times higher than superinduced fibroblasts) was obtained after amplification of the transgene to more than 50 copies per cell by coselection in methotrexate for dihydrofolate reductase gene amplification. The IFN-β, which can be purified from culture medium harvested twice per day, is a glycoprotein of 22 kDa identical to the natural molecule in its polypeptide sequence and in its high specific activity of more than $3x10^8$ IU/mg protein. CHO producer clones are genetically stable and suitable for large-scale, high-density continous cultures in microcarrier fermentor systems,which can be harvested several times a day for months giving yield efficiencies 1,000 times higher than the discontinuous fibroblast superinduction systems. Development of the constitutive CHO IFN-β production was one of the first cases in which mammalian recombinant cell technology could be shown to be competitive in efficiency to bacterial production systems. The carbohydrate structure of IFN-β produced in CHO cells has the high ratio of bi- to triantennary glycosyl chains which is the most similar to that of natural IFN-β (Figure 1) although with slight differences in the exact percentage of chains and sialic acid as revealed by analysis of various CHO-IFN-β preparations (Conrad et al., 1987; Utsumi et al., 1989; Fierlbeck et al.,1994).

C. Recombinant IFN-β from *E.coli*: The Betaser 17 Mutein

The presence of an unpaired cysteine residue (Cys^{17}) in addition to one disulfide bridge (Cys^{31}-Cys^{141}) in human IFN-β can cause the formation of wrongly folded molecules, particularly when the protein is produced in *E.coli* bacteria which lack proper sulfhydryl exchange enzymes, and such abnormal disulfide bonds were observed experimentally (Kimura et al., 1988). Anticipating that the additional Cys17 may

Table 1. Properties of Various Human IFN-β Preparations

Preparation/Property	*Betaseron*	*Native IFN-β (Frone)*	*Recombinant IFN-β (REBIF)*
Production System	*Escherichia Coli* recombinant bacterial	Human fibroblasts natural human	Hamster CHO cells recombinant mammalian
Primary Structure	165 aa Serine instead of Cysteine at #17 Lacks Methionine #1	166 aa N-terminal Methionine	166 aa As Native
Molecular Weight	18.5 Kd	22-24 Kd	22-24 Kd
Carbohydrates	No	Yes	Yes
Bioactivity *In Vitro*	3.2×10^7 IU/mg	$>3 \times 10^8$IU/mg	$>3 \times 10^8$IU/mg
Dose for 2 x OASE *In Vivo*	3 MU	1.5 MU	1.5 MU
Antigenicity	High	Low	Low

be the reason for the low specific activity yields and insolubility of IFN-β in *E.coli*, Mark et al. (1984) replaced this residue by a serine codon in the IFN-β cDNA. The resulting molecule IFN-βser17 produced in *E. Coli* had the anti-viral, anti-growth, and NK activation properties of natural IFN-β (Mark et al., 1984) and its improved stability allowed to develop industrial production of this product known as betaseron or IFN-β1b (vs. IFN-β1a for the CHO-product). In addition to being unglycosylated, betaseron is 165 amino acid long (lacking the N-terminal methionine of the natural or CHO-IFN-β) and these differences probably explain why betaseron is somewhat different immunologically from fibroblast or CHO-IFN-β (Colby et al., 1984). In all publications prior to April 1993, the bioactivity of betaseron seems to have been expressed in standart units which were not comparable to those used for natural or CHO-IFN-β: when now standardized to be in line with other IFNs, what was previously 45 million units should now becomes 8 million units or a reduction of 5.6 fold in the nominal dose as stated by The IFNβ Multiple Sclerosis Study Group (1993).

Table 1 summarizes the properties of three main forms of IFN-β available for clinical usage, taking into account the revised specific activity of betaseron as compared to the glycosylated forms. The *in vivo*

dose needed for a 2'-5' A synthetase (OASE) increase and antigenicity are discussed in the following sections.

VI. PHARMACODYNAMICS OF IFN-β AND SIDE EFFECTS IN MAN

A. Pharmacokinetics and Pharmacodynamics

There is an important difference in the pharmacokinetics of IFN-β as compared to leukocyte IFN-α or to rIFN-αA (Billiau, 1981; Bocci, 1992). Upon injections of natural IFN-β by the intramuscular or subcutaneous (i.m, s.c.) routes, the level of protein in the blood circulation remains low and is about 10% of that observed with IFN-α (Hawkins et al., 1984; Paulesu et al., 1992; Bornemann et al., 1985). However, IFN-β is active when administered by i.m. or s.c. routes, and experimental data show that IFN-β is adsorbed and diffuses through the lymphatic system (Bocci et al., 1988). During its transit through the lymph ducts and nodes, IFN-β reaches a great number of effector cells and tissues, before slowly emerging in the plasma at 5-10 h. The bioactivity of natural IFN-β injected i.m. or s.c. is demonstrated by pharmacodynamic tests which measure the response of white blood cells by quantifying the increase of IFN-dependent 2'-5' A synthetase (Revel et al., 1981; Schattner et al., 1981; Schonfeld et al., 1984). Cell or serum assay of this and other markers such as the IFN-dependent Mx protein, or neopterin resulting from GTP metabolism in activated monocytes, showed similar increases following i.m. injections of either natural or CHO-produced recombinant IFN-β (Liberati et al., 1992). These increases are large (4-8-fold), observed from 6 h until 72 h after single injections but highly sustained when IFN-β is given every other day; they are dose dependent at 3- 9 million units (MIU), and correlate with the anti-viral state of the white blood cells and with natural killer (NK) cell activity (Schonfeld et al., 1984; Liberati et al., 1992). Sustained elevation of 2'-5' A synthetase during 24 weeks of CHO-IFN-β treatment was reported (Fierlbeck et al., 1994). Comparable biological response of such markers and of NK cells is observed after s.c. injections of *E.coli*-produced IFN-βser, again in the virtual absence of IFN-β in the serum (Goldstein et al., 1989). The same work with high doses of s.c. rIFN-βser (8-32 MIU after correction) showed an increase of blood cell surface MHC antigens and of β2-microglobulin (β2m) serum level. The immunological significance of serum β2m which denotes shedding of β2m from cells into the plasma,

is unclear but shedding was also reported at low dose rIFN-βser (Witt et al., 1993) and with IFN-α (Lucero et al.,1982). An increase in β2m on cells but no shedding was observed with glycosylated IFN-β at 6-10 million units i.m. (Lucero et al., 1982; Liberati et al.,1992), the serum β2m increase being observed after intravenous (i.v.) infusion of the natural or CHO-IFN-β (Liberati et al., 1988,1994b) as well as during prolonged treatment (Fierlbeck et al., 1994).

Serum levels of IFN-β are measurable after i.v. injections of natural IFN-β or rIFN-βser and indicate similar pharmacokinetics with a fast initial half-life of 5-15 and 20 min followed by terminal half-lifes of 4-5 h respectively (Liberati et al., 1989; Chiang et al., 1993). Even by the i.v. route, IFN-β initially leaves the blood stream faster than IFN-α, in line with its higher uptake in other tissues (Bocci, 1992). The carbohydrates of IFN-β, which vary slightly between mammalian preparations may influence tissue distribution as a result of liver uptake and catabolism (Bocci et al., 1982; Utsumi et al., 1991). Nevertheless, the pharmacodynamic response parameters show grossly comparable inductions with the different human, mammalian, or bacterial IFN-β preparations given by i.v. bolus or perfusion (Liberati et al., 1988, 1991, 1994b; Goldstein et al., 1989; Borden et al., 1990). When the i.m. and i.v. routes are compared for one IFN-β at the same dose (6 million units), the biological responses are of comparable intensities but of shorter duration in the i.v. than the i.m. route (Liberati et al, 1994b). Since the pharmacodynamic markers, for example increase in 2'-5' A synthetase, often correlate with clinical responses (Giannelli et al., 1993; Rusconi et al., 1994), there may be no benefit in giving IFN-β by i.v. rather than by the i.m or s.c. routes. Bocci (1992) warns that maintaining high IFN-α or IFN-β levels in the plasma may not be advantageous, because this merely increases the catabolytic loss of IFN through liver and renal processing, and increases the toxicity and side effects of IFN. In fact, the slow diffusion and adsorption of IFN may have some advantage, the differing pharmacokinetic profile of IFN-β as compared to IFN-α even contributing to the milder side-effects which have been observed with IFN-β in clinical trials (see following).

By the pharmacodynamic parameters, IFN-β injected i.m. or s.c. appears as active as IFN-α despite the difference in their blood levels. Thus, measurement of IFN-induced enzymatic activities such as the 2'-5' A synthetase (OASE) provides a method of comparing the in vivo efficacy of different IFNs. Comparison of published data for natural or CHO-produced IFN-β and for *E.coli*-produced rIFN-βser (after correction of units) indicates that the dose needed to achieve a two-fold increase

in this enzyme extrapolates to 1.5 and 3 million units, respectively (see Table 1).

B. Side Effects and Toxicity

Type I IFN therapy is associated with a number of side effects some of which reflect the biochemical changes that are part of the IFN response in various tissues. The acute effects following IFN administration are "flu-like" symptoms which are typical of viral infections inducing IFN in the body, and through it prostaglandin formation and leucocyte mobilization (Yaron et al., 1977; Schattner et al., 1982). These acute or early effects can include fever, headache, myalgia, tachycardia, hypo- or hypertension, and gastrointestinal disorders which can be alleviated by anti-inflammatory drugs and tend to disappear when IFN is given every other day. More serious are chronic side effects; among them are neurological toxicity, cardiovascular accidents, or auto immune thyroiditis which can limit treatment especially with IFN-α as recently reviewed (Quesada, 1992; Vial and Descotes, 1994). IFN-β also produces flu-like symptomes but the side effects are in general milder and fewer than IFN-α2 (Nagai, 1988) as illustrated in the comparative compilation of adverse effects in Table 2. In particular, hematological depression is less marked with IFN-β and there is no depression of the erythropoiesis (anemia) as observed with IFN-α, a difference which is also apparent in BM cell colony assays *in vitro* (Michalevicz and Revel, 1987; Michalevicz et al., 1988). IFN-β has low hematological toxicity even when combined with IL-2 (De Braud et al., 1994). No signs of autoimmune thyroiditis were seen in patients treated with six MIU i.v. natural IFN-β for up to 6 months (Pagliaci et al., 1991).

IFN-β produces fewer disturbances of the central nervous system such as asthenia, depression, and behavioral and cognitive changes which develop during chronic IFN-α therapy and which largely contribute to the negative influence on quality of life reported in up to 50% of patients treated with 9 MIU IFN-α (Grion et al., 1994). Psychiatric complications of long-term IFN-α therapy were described (Renault et al., 1987). In a recent study in Japan comparing Hepatitis C patients treated with either IFN-α or IFN-β, subclinical abnormalities scored by a self-depression scale were more increased by IFN-α than by IFN-β (Matsushita et al., 1994). Infusions of IFN-β at 6 MIU/m2 i.v. for seven days or longer (alternate weeks) showed no significant changes in EEG, visual evoked potentials, sensory or motor nerve conduction, and neuropsychological monitoring

Table 2. Side Effects of Interferons

Side Effects		*IFN-β*	*IFN-α*
Flu-like symptoms	Fever	f (50-90)	f (90
	Myalgia	<f (25)	f (50)
	Headache	s (20)	s (20)
	Chills	s (15)	s (20)
	Lassitude	s	s (20)
Digestive symptoms	Nausea, Vomiting	n (3)	f (30)
	Diarrhea	s	s (10)
	Anorexia, Weight loss	s	f (50)
Hematological depression	Leucopenia	<f (20-65)	f (90)
	Thrombocytopenia	s	f (50)
	Anemia	n	f (40)
Neurotoxicity	CNS disturbance	n	s
	Depression	n	s
	Dementia	n	s
	Weakness, Lassitude	s	s (20)
Skin	Hair loss	n	s
	Psoriasis	n	s
Hepatic dysfunction	Aminotransferase elevation	s	f
	Autoimmune	n	s
	Drug metabolism decrease	n	s
Endocrine	Autoimmune thyroiditis	n	s
Cardiovascular disorders	Infarction	n	s
	Arrhythmia	n	s
	Hyper,hypotension	s	s
Respiratory diseases	Interstitial pneumonia	n	s

Source: Adapted from Nagai (1988), and a Serono review of Japan registration forms for IFNs.
Notes: f=frequent, <f=less frequent, s=sometime, n= rare or not observed. Numbers in parenthesis are a percentage of cases showing side effects.

failed to disclose IFN-β-induced deterioration (Liberati et al., 1990a). This contrasts with changes in EEG, behavior, and cognition observed with IFN-α treatments (Adams et al., 1984; Mattson et al., 1984). IFN-α infusion over five days produces significant deterioration of verbal memory, calculation, and writing ability, which improve after cessation of treatment showing IFN-α effect on CNS functions (Poutanien et al., 1994). IFN therapy is often associated with somnolence and human rIFN-α2 has

been found to be somnogenic in rabbits when injected in the cerebral ventricules; in the case of human rIFN-β, it appeared that these effects were due to contaminants rather than to IFN-β itself (Kimura et al., 1994). However, transient somnolence, and mild fatigue are seen with rIFN-β as well in 35% of cases (Liberati et al., 1992). Hearing disabilities have been noted with both IFN-α and IFN-β which resolved at the end of treatment (Kanda et al., 1994). Nevertheless, IFN-βser has beneficial effects on ear functions in colds (Sperber et al., 1992) and, in general, is effective in treating neurological diseases (see Section VIIA).

The side effects and particularly the neurotoxicities vary in severity and onset with the IFN dosage, often imposing a limit to treatment. With IFN-α, doses of > 20MIU produce severe fatigue and other limiting side effects (Quesada, 1992). Phase I and therapeutical uses of natural and CHO-IFN-β indicate that the maximal tolerated dose (MTD) is more than 40 MIU (Liberati et al., 1989, 1992, unpublished). For the *E.coli*-produced rIFN-βser, the MTD is >30 MIU (standardized units, Yung et al., 1991) but rIFN-βser was seen to produce unacceptable toxicity at 16 MIU in a recent trial (The IFN-β Multiple Sclerosis Study Group, 1993). It is tempting to speculate that the reason for the good tolerance and milder side effects of the glycosylated IFN-β may be related to the fact that natural IFN-β is the type produced by most tissues early in viral diseases, or may be related to its preferential diffusion through the lymphatic route rather than through the blood circulation (see Sections IVG and VIA).

C. Antibody Reactions in Treated Patients

Repeated injections of proteins, in particular recombinant modified proteins, has the inherent danger of causing formation of neutralizing antibodies. This problem is also encountered in the clinical uses of various IFNs and the significance of such antibodies has been discussed (VonWussow and Borden, 1989). Recombinant *E. coli*-produced IFN-α2a used in the treatment of chronic hepatitis C induces neutralizing antibodies in 50% of patients in 12 months and this partly correlates with the failure to respond to treatment by aminotransferase normalization (Milella et al., 1993; Bonetti et al., 1994). Anti-rIFN-α2a antibodies from patients can neutralize multiple IFN-α subtypes from natural sources (Brand et al., 1993). Since this can impair the patient's own IFN- α response, use of natural or rIFN-α2c which are less immunogenic is important. There is no immunological cross-reaction of IFN-α with

IFN-β, but IFN-β preparations can induce their own antibodies which would neutralize endogenous IFN-β. Thus, high frequency (55-95%) of neutralizing antibodies were reported in melanoma patients treated with fibroblast-derived IFN-β given at 3 MIU s.c. for six months but this preparation was only 10% pure and may have contained other cytokines (Dummer et al., 1991; Fierlbeck et al., 1994). In the same studies, CHO-produced IFN-β gave rise to antibodies in only 28% of cases. In a comparative study, rIFN-α2a produced antibodies but not natural IFN-a or IFN-β even using a Western blot that would detect also non-neutralizing antibodies (Arai et al., 1994). A study of 45 patients treated with highly purified natural IFN-β 3-6 MIU i.m for up to six months showed less than 9% cases with neutralizing antibodies (Fernandez et al., 1995). Data on rIFN-βser indicate that i.v. injections lead to around 17% of neutralizing antibodies but s.c. injections of 1.6 or 8 MIU every other day for a year gave 45% incidence of neutralizing antibodies (The IFN-β Multiple Sclerosis Study Group, 1993). This rather high incidence may be related to the 10-times lower specific activity of this rIFN-βser as compared to natural or CHO glycosylated IFN-β (Table 1). This stresses the importance of using pure and high specific activity IFN-β as similar as possible to the endogenous IFN-β which will minimize the formation of neutralizing antibodies.

D. Alternative Routes of Administration

Intranasal application or spray of IFN have been studied in the context of experimental rhinovirus colds. While protecting against rhinovirus infection, IFN-α produced a dose-dependent increase in local reactions including nasal stuffiness, blood tinged mucus, and mucosal erosions with subepithelial lymphocytic infiltration (Hayden et al., 1985). rIFN-βser appears to cause fewer side effects when administered in the nasal mucosa even for 25 days, giving only minimal signs of nasal irritation, while showing reduction of viral clinical colds (Higgins et al., 1986; Hayden et al., 1987). Hence, IFN-β could show a more favorable therapeutic index than IFN-α. Inhalation of IFN is another route being experimented with in patients with lung cancer: IFN-β appears to be better tolerated than inhaled IFN-α in comparative studies aimed at delivering IFN to the alveolar epithelial lining of the lungs (Halme et al., 1994).

Dermal ointments of IFN-β, either in gels or creams, are topical treatments for recurrent herpes simplex labialis and genitalis which are effective in reducing the rate of recurrence in addition to alleviating

eruption of symptomes in a safe and well tolerated way (Glezerman et al., 1988; Ophir et al.,1995). Occular drops of IFN-β have been used effectively in treatment of adenoviral epidemic keratoconjunctivitis (Romano et al., 1980) and herpes simplex keratoconjunctivitis (Vannini et al., 1986a, 1988b; Romano and Sadan, 1988). These dermal and occular formulations contain about 0.1 MIU/g and are most efficient with 4-6 applications per day.

IFN-β has been injected safely and effectively intrathecally into the cerebrospinal fluid of multiple sclerosis patients (Jacobs et al., 1986). Systemic treatments by s.c. or i.m. injections of IFN-β are now being used in this indication (Section VII). Animal experiments show that IFN-β given by i.v. reaches the brain in particular in mice with glioma tumors (Mihara et al., 1991; Wiranovska et al., 1994). IFN-β has also been injected intralesionally in papilloma virus lesions of the genitals and cervix (Bornstein et al., 1993a) and into tumors such as melanoma nodules (Rosso et al., 1985). Intrapleural injections of IFN-β were used to control malignant pleural effusions (Rosso et al., 1988).

Oral administration of IFN-β is being experimented with in mice to induce immunoregulation through intestinal immune cells (Brod and Burns, 1994). Oral administration does not seem to induce systemic biological effects (Witt et al., 1992), but this may be achieved by special coating and gut-uptake devices in the future.

VII. MAJOR CLINICAL APPLICATIONS OF IFN-β: INFLAMMATORY AND VIRAL DISEASES

The biological activities of IFN-β as immunoregulator, antiproliferative, and antiviral agent (see Section III) have led to clinical trials in autoimmunedysfunctions, viral diseases, and malignancies. The present status of IFN-β therapy, alone or in combination with other treatments, is reviewed for selected applications together with some insights on the mechanism of action involved.

A. Multiple Sclerosis

Clinical Trials in Relapsing-remitting MS Trials with natural fibroblast IFN-β administered intrathecally were initiated in the early 1980s by Jacobs et al. (1981) for the treatment of relapsing remitting multiple sclerosis (MS). The initial rationale was that viral infections may act as triggers for the repeated exacerbations of neurological symp-

toms characterizing MS, but it is now much more likely that the main effect of IFN-β is an immunoregulatory action inhibiting the autoimmune activation of T cells which underlies the MS demyelination process (see the following section). These early trials (Jacobs et al., 1986) showed that natural fibroblast IFN significantly reduced the exacerbation rates but the administration of IFN-β by the intrathecal route (once a month) was not very practical and potentially hazardous.

The efficacy of systemic IFN-β in multiple sclerosis was established in a double-blind placebo controlled study of 120 patients/ followed for 2-3 years (The IFN-β Multiple Sclerosis Study Group, 1993). The Betaseron IFN-β-1b used in this study was administered at 1.6 or at 8 MIU subcutaneously every other day to one group and the other received a placebo. Over two years there was, at the 8MIU dose, a 34% decrease in the rate of exacerbations observed ($p = 0.001$) and a 49% decrease in severe exacerbations. The percentage of patients free of exacerbation increased twofold from 18 to 36% ($p = 0.007$). Relevant to the cost effectiveness of the treatment is the fact that the hospitalization days over three years decreased by 43% for those receiving the treatment as compared to those receiving the placebo. The lower dose of IFN gave only small decrease in these different parameters. This study also did not show a significant change in disability which worsened in 27% of the patients treated with 8 MIU IFN-β-1b versus 39% of those receiving the placebo. However, the most striking effect of IFN-β therapy was on the number of lesions measured by magnetic resonance imaging (Paty et al.,1993). Thus, in a group of 52 patients who were scanned by MRI every six weeks during the two years, there was a median decrease of 83% in active lesions per patient, per year ($p = 0.03$) and a 75% decrease in new lesions ($p < 0.003$). From data available for about 110 patients in each group, there was a 9.3% median decrease in total lesion area in the group receiving the higher IFN dose as compared to a 15% increase in the placebo group. The difference between the treated and placebo became already apparent at six weeks. Although the MRI lesions probably reflect more the inflammatory process during exacerbations than the demyelination process causing the neurological deficiency, these MRI results show that IFN-β clearly has an objective effect on the disease (Paty et al., 1994). Side effects of the IFN-β therapy were mild as usual, but the percentage of patients developing antibodies to the Betaseron mutein was relatively high, 45% developing neutralizing antibodies at some point; sustained neutralizing antibodies were observed in 27, 33, and 38% of patients at one, two, and three years, respectively (The IFN-β

Multiple Sclerosis Study Group, 1993, 1995). Six-year follow-up of such treatments was reported (Knobler et al., 1993).

In phase III trial with CHO produced recombinant IFN-β, about 300 patients with relapsing remitting MS received rIFN-β-1a, 6 MIU intramuscularly once a week for two years, or a placebo in a controlled double blind setting (Jacobs et al., 1996). A more important clinical end point was used for this trial which is the progression of disability by one point on the Kurtke Extended Disability Status Scale (EDSS). At two years 34.9% of the placebo patients versus 21.9% of the IFN-β-1a treated patients had deteriorated by one EDSS point (37% decease, p=0.02), indicating a clinical beneficial effect of IFN-β. The median time progression was increased by 75% (p=0.03) in the IFN-β group (from 3.08 years to 5.42 years). Exacerbations per year were reduced by 32% (p=0.002) and the number of patients with three or more attacks/year was reduced from 31 to 14% (55%). The side effects were modest and neutralizing antibodies were seen in only 14 and 22% at one and two years, respectively. Considering the lower dose/week than that used in the Betaserson trial, the clinical efficacy of CHO rIFN-β-1a appears good in this trial (Jacobs et al., 1996). In another trial with CHO rIFN-β-1a given subcutaneously three times per week to 72 relapsing remitting MS patients in a pre-test/post-test design, decreases in the exacerbation rates of 53% and 69% (p=0.007) were seen at the doses of 3 and 9 MIU, respectively. By MRI, the new lesions were decreased by 65 and 70%, respectively (p<0.001) (Pozzilli et al., 1996). Natural IFN-β given subcutaneously (9MIU, 3x/w) was also active in a trial comparing 30 treated patients to untreated patients; the number of active lesions by MRI decreased 58-67% (p<0.05) and mild side effects such as flue-like symptoms were seen in only 36% of patients (Fernandez et al., 1995).

In several trials, IFN-α did not produce statistically significant clinical effects similar to those of IFN-β in multiple sclerosis (Panitch and Bever, 1993). A recent small trial with high dose rIFN-α2a indicates reduction in MRI lesions and in IFN-γ secretion from lymphocytes, but creates more side effects (Durelli et al.,1994).

Mechanism of Action of IFN-β in Multiple Sclerosis

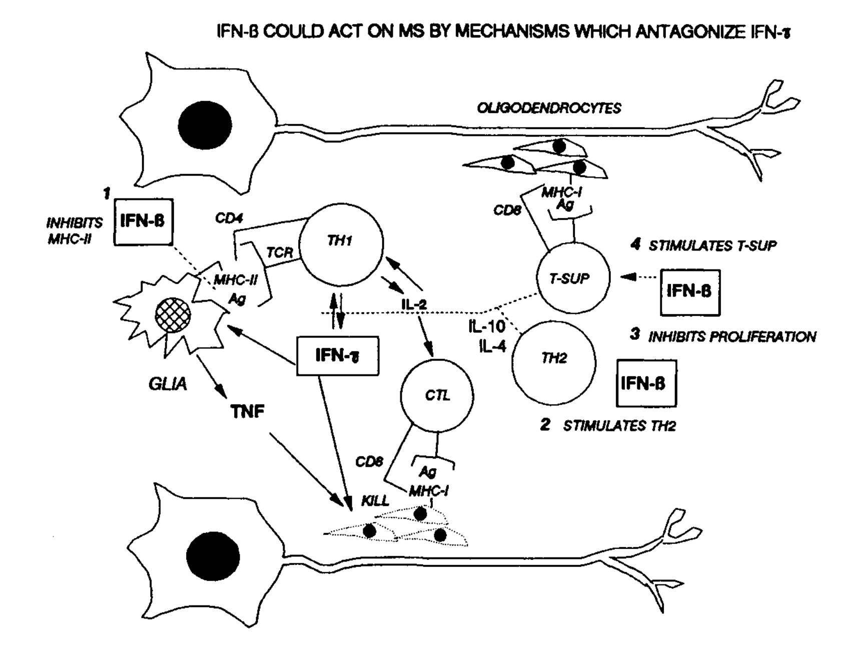
The present concept of MS implicates an autoimmune process due to activation of T cells by some myelin antigen (e.g., peptides of the myelin basic protein) presented by Major Histocompatibility class II (MHC-II) molecules on the surface of astrocytes, microglia, and endothelial cells

in the brain (Wucherpfennig et al.,1991; Panitch and Bever, 1993). IFN-γ is a primary up-regulator of MHC-II expression on cells (Basham and Merigan, 1983; Rosa et al., 1986) and appears with MHC-II expressing astrocytes and macrophages at the sites of active demyelination in chronic active MS plaques (Traugott and Lebon, 1988). On endothelial cells, including those lining brain microvessels, IFN-γ increases MHC-II and antigen presentation to circulating $CD4^+$ T-lymphocytes, as well as enhances lymphocyte adhesion and transmigration through the blood-brain barrier (Huynh and Dorovini-Zis, 1993). IFN-γ is a strong activator of macrophages (Talmadge et al., 1986) which participate in the demyelination process and killing of oligodendrocytes (Bever and Whitaker, 1985). IFN-γ-activated macrophages or glial cells produce proteases and TNF-α which has a cytotoxic effect on oligodendrocytes *in vitro* (Selmaj and Raines, 1988). IFN-γ synergizes with TNF to induce MHC-II and augment the cytotoxic effects and high TNF production by blood cells *in vitro* can predict relapses (Chofflon et al.,1992), TNF levels in CSF of MS patients correlating with clinical severity (Sharief and Thompson, 1992). Measurements of IFN-γ give more variable results (Chofflon et al., 1992) but higher than normal IFN-γ serum levels are observed (Revel et al., 1995). Since injections of IFN-γ to relapsing-remitting MS patients caused an increased incidence of exacerbations, there is strong evidence that IFN-γ acts as a mediator of MS attacks (Panitch et al.,1987). Increases in IFN-γ may result from intermittent infections (Panitch, 1994) but also from the activation of T cells by the autoimmune trigger. IFN-γ secretion is a property of $CD4^+$ TH1-helper lymphocytes mediating cellular immunity, whereas TH2 clones produce IL-4 and IL-10 and inhibit the TH1 IFN-γ production (Mosmann and Moore, 1991). Activation of TH1 clones by brain or microvessel cells presenting antigens through MHC-II can cause a loop whereby IFN-γ enhances antigen presentation which in turn promotes TH1 secretion of IFN-γ with inhibition of TH2 functions, which could account for the decreased suppressor T cell activity noted in MS (Noronha et al.,1992).

IFN-β could act on several of these cytokine triggers of MS exacerbations, particularly those related to IFN-γ. Thus, injections of natural IFN-β (3 MIU i.m., 3x/w) to relapsing-remitting MS patients was shown to cause a reduction in their elevated IFN-γ serum levels (Revel et al., 1995). The reduction was already significant one month after the onset of treatment and was sustained for the six months of treatment whereas the IL-4 levels tended to increase-IFN-β strongly stimulates IL-10 secretion by monocytes *in vitro* (Porrini et al., 1995). IFN-β may,

therefore, restore a more normal TH1 to TH2 functional ratio. The low suppressor T cell activity of MS patients seems also to be reverted by IFN-β (Noronha et al., 1992; Panitch and Bever, 1993). The mechanisms by which IFN-β could enhance suppressor activity remain unclear but such effects would interfere with IFN-γ production and reduce the autoimmune process (Figure 6a, squares 2-4). From the clinical evidence (Panitch, 1994), it does not appear that IFN-β reduces IFN-γ by decreasing the intermittent infections which in MS patients correlate with exacerbations, but rather by an immunoregulatory effect.

An immunoregulatory action of IFN-β which is better understood at the molecular level is the inhibition of MHC-II induction by IFN-γ. This occurs in brain glial cells, astrocytes or gliomas (Joseph et al., 1988; Barna et al., 1989) through a transcriptional effect (Devajyothi et al., 1993) and is observed also in endothelial and epithelial cells (Revel et al., 1995; Miller et al., 1995). The induction of the HLA-DR gene expression is a secondary response to IFN-γ, probably resulting from the synthesis of a transactivator such as the CIITA factor (Steimle et al., 1994) acting together with the RFX-1 protein which binds to the X-box in the HLA-DR promoter (Siegrist and Mach, 1993). The CIITA gene appears to be under the control of the IFN-γ receptor primary intracellular signals (Steimle et al., 1994) which involve activation of Jak1/Jak2 tyrosine kinases and of



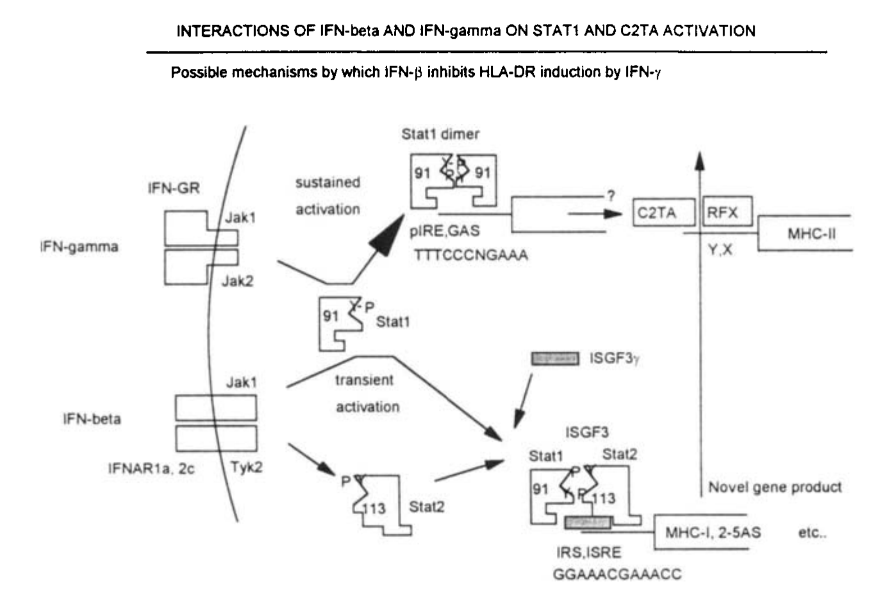


Figure 6. (A) Pathogenic functions of IFN-γ in exacerbations of relapsing-remitting Multiple Sclerosis underlying the demyelination process. Possible sites of action of IFN-β are shown as discussed in the text. (B) Model of IFN-β interference with the Stat1 activation pathway and with the action of the CIITA transcription factor through which IFN-γ induces major histocompatibility antigens of class II (MHC-II).

the Stat1 transcription factor binding IFN-γ activatable sequences (GAS) of primary IFN-γ responsive genes (Darnell et al., 1994). IFN-α and β also activate Stat1 but in this case Stat1 becomes part of the ISGF3 complex (Stat1, Stat2, and ISGF3γ) which binds to the ISRE elements such as found in MHC-I genes (see Section II, Figure 3). The observation that IFN-β inhibits the IFN-γ dependent activation of Stat1 binding to GAS DNA elements allows to propose a model (Revel et al., 1995; Figure 6b) explaining the antagonism of IFN-β against IFN-γ for MHC-II gene induction while accounting for their synergistic effect on ISRE-mediated MHC-I induction (Vaiman et al., 1990). Thus, in the presence of IFN-β the Stat1 activity becomes transient, eliciting ISGF3 formation but no accumulation of free activated Stat1 as normally occurs in response to IFN-γ, thereby preventing induction of the transactivator of MHC-II transcription (Figure 6b). Hence, Stat1/GAS mediated effects of IFN-γ would be inhibited by IFN-β, whereas other effects which may not be

strictly Stat1-dependent would not be blocked by IFN-β such as ICAM-1 induction (Satoh et al., 1995; Miller et al., 1996) or secretion of IL-1 or TNF by IFN-γ activated monocytes (Porrini and Reder, 1994). An effect of IFN-β on the action of the CIITA transcription factor was shown to occur downstream of the CIITA gene induction, suggesting the IFN-β may induce a protein inhibiting CIITA (Lu et al., 1996). Considering the crucial role of MHC-II in the antigen presentation by glial or endothelial brain cells in MS, this interference of IFN-β or IFN-γ and CIITA could be the basic mechanims by which IFN-β prevents the increased autoantigen presentation to CD4+ T cells and overcomes spurring of exacerbations by IFN-γ (Figure 6a, square 1). In view of the higher sensitivity of glioma cells to IFN-β than to IFN-α (Rosenblum et al., 1990), the brain microglia may be particularly sensitive to this anti-IFN-γ action of IFN-β inhibiting MS exacerbations.

Another immunoregulatory activity may be related to the inhibition of T cell proliferation by IFN-β (Figure 6a, square 3). Peripheral blood mononuclear cells treated by IFN-β *in vitro* and *in vivo* show decreased Con A-driven proliferation and IL-2R expression (Rudick et al., 1993). An interesting observation is that type I IFNs can inhibit the proliferative response of T cells to superantigens, such as staphylococcal enterotoxins, whereas IFN-γ does not (Soos and Johnson, 1995). Bacterial superantigens are believed to be among the triggers of autoimmune attacks, and the inhibitory effect of IFN-β may well be another protective mechanism against such immunological shocks.

The location of IFN-β action in MS is not clear: Some IFN given systemically may reach the brain MS lesions if the blood-brain barrier is breached (Mihara et al., 1991; Wiranowska et al., 1994), but IFN may also act on the endothelial cells of brain microvessels or on immunocytes circulating in blood or lymph. In the experimental autoimmune encephalomyelitis (EAE) murine model, oral feeding of type I IFN was quite effective in decreasing the T cell response to myelin protein antigens and CNS inflammation indicating an effect at a distance (Brod and Burns, 1994). Whether oral IFN could be administered in human MS is a challenging possibility, but one has to remember that EAE is only a partial model to MS where, for example, IFN-γ has beneficiary effects instead of the pathogenic effect seen in man (Panitch and Bever, 1993). The action of IFN-β in the autoimmune MS pathology suggests that IFN-β may be useful in other demyelinating diseases, such as, for example, Adrenoleukodistrophy, a notion supported by pilot treatements (Moviglia, personal communication).

B. Papillomavirus Genital Lesions

Condylomas

Condyloma acuminata, genital warts associated mainly with human papillomavirus (HPV) subtypes 6 and 11, is a sexually transmitted disease (STD) of increasing frequency (Oriel, 1983; Schneider et al., 1987). Ablation of the warts by laser surgery, or by chemicals, has the drawback of causing pain and scars, and leaving behind infected cells from which the warts can regrow leading to a high rate of recurrence. Interferon can cause elimination of resident episomal papillomavirus in experimentally transfected cells (Turek et al., 1982). Several studies indicated that IFN-β is an effective treatment producing the long-term elimination of condylomas with a reasonably high rate of success. Olmos et al. (1994) recently reported a large randomized, double-blind, placebo-controlled trial of 100 patients, with a mean of five lesions per patient. A 10-day course of 2 MIU/day natural IFN-β given systemically by the intramuscular route resulted in 51% complete response defined as total disappearance of all condylomatous lesions, versus 28.9% in the placebo group. The lesions typically disappear after about six weeks, which is probably the time for epidermal replacement following viral elimination. No recurrence of the lesions was observed after a year. There were not more reported side effects with the IFN-β treatment than with the placebo which may have some psychological effect in this disease. The results of this trial confirm previous smaller trials with similar short courses of systemic IFN-β which resulted in warts disappearance in up to 80 % of cases (Schonfeld et al., 1984; Costa et al., 1988). Genital HPV lesions such as condylomata are frequent in HIV-positive immunodeficient patients but still respond well to IFN-β or α treatments. In a recent study, intramuscular administration of natural IFN-β 3MIU every other day for 15 days produced, at six months, a cure rate of HPV genital warts of 89% in HIV-negative and of 50% in HIV-positive patients (Semprini et al., 1994). Intralesional (i.l) injections of IFN-β or α also show efficacy compared to placebo (although i.l. placebo can reduce the warts), but the i.l. route does not give higher cure rates than the systemic injections and seems to have little effect on neighboring non-injected warts (Reichman et al., 1988; Welander et al., 1990). Intralesional administration is time consuming and difficult in patients with multiple lesions, and hence may be of interest only in large anogenital warts. Indicators of IFN action such as the 2'-5' A synthetase serum level, which increases markedly

after systemic injection of IFN-β, may be predictive of the clinical response in genital HPV: women with elevated baseline 2'-5' A synthetase prior to IFN-β therapy showed much less response than women with more pronounced increases from pre to post IFN-β injection (Rusconi et al., 1994).

Cervical Neoplasia and Vulvar Lesions

Cervical intraepithelial neoplasia (CIN I-III) associated with HPV subtypes 16,18, together sometimes with 6,11 (Schneider et al., 1987) appear to respond to IFN therapy. The lesions caused by HPV 16,18 which integrate in the genome of transformed cells are more resistant than those of HPV 6,11 but long-term remission can nevertheless be achieved. An immunological effect of IFN may contribute to eliminate the virus-transformed cells since response correlates with NK cell increases (Garzetti et al., 1994). A review of several placebo controlled clinical trials (Bornstein et al., 1993a) suggests that systemic injections of IFN-β may give the best results in CIN-II patients with disappearance of the lesions (CR) in 80-90% of cases versus 40% or less with placebo. CR are less frequent in CIN-III than in CIN-II (Costa et al., 1988). Following a 10 day treatment with i.m. IFN-β, long-term regressions (2 years) were observed in more than one-third of CIN-II patients versus none with the placebo (De Aloysio et al., 1994). In other trials, intralesional injections of IFN-β were made repeatedly into the cervical dysplasia, which also yielded 55-80% of CR in CIN-II or III, whereas vulvar neoplasia VIN-III were more resistant (DePalo et al., 1990; Penna et al., 1994). Moreover, topical applications of IFN-β alone or with cis-retinoic acid on HPV lesions of the uterine cervix were also reported to yield 88% and 94% response rates respectively in a group of 130 women (Markowska et al., 1994). Many variations, however, have been observed in different clinical trials for CIN, and in several studies with IFN-α there have been low response rates (see Bornstein et al., 1993a for review). A large trial with low s.c. doses of natural IFN-α did not show a difference with placebo (Yliskoski et al., 1991) and similar low s.c. doses of rIFN-α -2a did not even affect condylomatous lesions (Condylomata International Collaborative Study Group, 1993). Invasive cervical carcinoma is not amenable to IFN treatment (Bornstein et al., 1993a). Therefore, in patients with advanced preneoplastic lesions (CIN-III), the benefits of IFN therapy will have to be weighed against surgical intervention. However, in patients with less advanced dysplastic cervical lesions,

IFN-β therapy seems to be a promising alternative which deserves further study.

Vulvar vestibulitis, another HPV-associated genital inaffection causing intense pain at intercourse (dyspareunia), responds to IFN therapy. Local administration of IFN can lead to resolution of the symptoms (Kent and Wisniewski, 1990) but is a painful treatment. Intramuscular injections of IFN-β at 5 MIU/day for a total of 2-3 weeks were effective in alleviating the symptoms for up to 18 months with few side effects (Bornstein et al., 1993b). In this HPV affection, IFN may again be a promising alternative to surgery.

C. Herpes Simplex Virus Recurrent Dermal Lesions

Pathogenesis of recurrent herpes labialis or genitalis, due to periodic reactivation of latent herpes simplex viruses (HSV) type I or II from ganglia neurons and their dermal replication, involves some deregulation of cellular immunity linked to hormonal and cytokine controls (Corey and Spear, 1986; Torseth and Merigan, 1986; Kuo and Lin, 1990). HSV latent infection is widespread and the recurrent forms of facial and genital herpetic eruptions affect a small percentage of the population in most countries causing severe discomfort, danger of virus spread to sexual partners or neonates, and cancer risk (Rapp, 1988). Genital herpes also favors AIDS virus transmission. As eradication of the latent virus has not been possible, treatment is aimed at preventing recurrences through inhibition of HSV replication and enhancement of the immune response. Acyclovir administered orally decreases the frequency of recurrent herpes but requires daily prophylactic treatment for years which should not be interrupted (Strauss et al., 1986; Kaplowitz et al., 1991) and does not work topically (Levin et al., 1989). These constraints and emergence of resistant HSV emphasize the interest for therapy that could be given topically and only during eruptions. Studies with IFN-β in repeated cream applications indicated its potential to reduce HSV titers in herpetic vesicles as well as the frequency of recurrences in labial and genital herpes (Movshovitz et al., 1985). Whereas trials with low doses of IFN-β have not shown efficacy (Batcheler et al., 1986), use of cream with 0.1 MIU/g in five daily applications for five days gave an average fivefold reduction in recurrences in 120 genital patients (Glezerman et al., 1988b). A double-blind, placebo-controlled trial with an IFN-β gel of similar stength gave a significant reduction of recurrences/year from 6.7 with placebo to 1.6 with IFN-β gel in labial and genital herpes patients

(Glezerman et al., 1988a). A more recent double-blind, placebo-controlled, randomized trial in 35 genital herpes patients showed that IFN-β cream, applied during eruptions (total dose 0.5 MIU), caused a significant reduction of recurrence/year from 10.3 to 3.5 compared with 9.7 to 6.9 by placebo, the recurrence rate with IFN-β being significantly lower ($p = 0.03$) than with placebo cream (Ophir et al., 1995). A fivefold decrease in recurrence rate was seen in 77% of patients. Decreased duration of lesions and alleviation of symptomes were also observed in these trials. The action of IFN-β is probably a combination of viral inhibition and immunoregulation. Increase of Langerhans cells, which play an important role in skin immunity, were reported in response to a cream of IFN-α (Ghersetich and Lotti, 1994). Interestingly, systemic injections of IFN-α have not been efficient in herpes (Levin et al., 1989) and only when associated with dimethyl sulfoxide in topical application, IFN-α produced a 1.9-fold reduction in recurrences compared to placebo (Shupack et al., 1992). At present, the fibroblastic IFN-β seems to be the topical agent most active against these recurrent viral skin lesions.

D. Hepatitis C and Hepatitis B

Acute Hepatitis C

Hepatitis C infections, sometimes occurring post-transfusion but often sporadic or asymptomatic, have the inherent risk of progression to chronicity with high frequency (Davis, 1990). With the advent of PCR technology to assess HCV RNA titers, it became possible to examine antiviral therapies even if symptoms are variable in these infections. IFN-β has been tested as a means to eliminate or reduce the HCV-RNA burden in patients following acute hepatitis. Omata et al (1991) showed that IFN-β at 3 MIU i.v. for five days and then 3 x/w for three weeks resulted in a 90% negativisation of HCV RNA compared to 8% in a control group. At one year, abnormal fluctuating transaminases (ALT) were seen in 93% of the controls and 36% of the IFN-β treated cases. Another study used 3 MIU IFN-β 3 x/w for four weeks with similar results (Ohnishi et al., 1991). In a dose-controlled study, HCV RNA resolution rates of >80% were obtained in the group receiving a total amount of 336 MIU IFN-β (Takano et al., 1994). The effect on HCV-RNA is likely to be clinically relevant since such patients with high serum HCV-RNA (>10^5 copies/ml) have >80% risk of chronic HepC,

whereas patients with less HCV-RNA burden are in most cases cured after the acute episode (Fukui et al., 1994).

In contrast to the strikingly positive results seen with IFN-β in resolution of acute hepatitis C, less activity has been observed with IFN-α (Viladomiu et al., 1992). Studies of the mode of action of IFN-β following acute hepatitis C, and prospective trials, may help establish this indication which has the potential to alleviate one of the main causes of chronic hepatitis and even reduce the risk of hepatocellular carcinoma (Tabor and Kabayashi, 1992).

Chronic Hepatitis C

IFN-α is widely used as first line treatment of chronic HepC (see Davis, 1990; Hoofnagle et al., 1993 for reviews). However, the proportion of patients who achieve normalization of transaminases is below 50% and sustained remissions occur in only 10-25% of cases, relapses occurring after treatment is discontinued. IFN therapy for 1 year seems to incur fewer relapses than six months (Carreno et al., 1993). Maintenance treatment may improve the outcome of IFN therapy, but at the cost of a decreased quality of life experienced in one-half the HepC patients treated by IFN-α (Grion, 1994). Hence, there is continued interest in testing IFN-β which may be better tolerated because of its lower side effects and may allow prolonged maintenance treatment.

A recent comparative survey in Japan indicates that HCV remission is usually obtained with IFN-α at 9 MIU daily for 2-4 weeks followed by 3 x/w for 22 weeks, whereas with IFN-β i.v. injections of 6 MIU/d for eight weeks are considered effective (Iino, 1994). The efficacy of IFN-β to reduce ALT was seen in a number of trials in which IFN-β was administered by i.v. infusions for 1-3 months, with 50-60% of patients showing sustained low ALT 3-6 months after the end of treatment (Arima et al., 1986; Nagashima et al., 1987; Kiyosawa et al., 1989). A comparison of patients with sustained ALT normalization to non-responders indicates that IFN-β produces high response rates in patients with low HCV RNA titers (responders having a mean titer of $0.4x10^4$/ml compared to $4x10^4$/ml for non-reponders), the genotype having somewhat less importance (Kobayashi et al., 1993). In a study of Italian patients with chronic hepatitis C, natural IFN-β 6 MIU/d by i.v. infusion, 6 x/w for two months also caused ALT normalization in five of eight cases and HCV RNA negativisation in six of eight cases, with minimal side effects (Chemello et al., 1995). However, there are still many unanswered

questions about IFN-β therapy such as what the long-term evolution of the disease is and what dosage is needed to achieve similar response levels by s.c. or i.m. administration. This dosage may be around 12 MIU or 18 MIU and the better tolerance of IFN-β may allow its use at high doses for long periods of time (Trepo, personal communication). Combinations of IFN-β with ribavirin may also give interesting results (Kakumu et al., 1993).

Parameters which would predict response to therapy would be usefull in chronic hepatitis. Measurements of the IFN-inducible enzyme 2'-5' oligoadenylate synthetase (2'-5' AS) in serum of chronic hepatitis C treated with rIFN-α 2b have shown that a marked sustained 2'-5'AS increase within one month after starting IFN, correlates with sustained ALT normalization at the end of six month therapy and at one year (Giannelli et al., 1993). On the other hand, when the 2'-5' AS increase is delayed, transient, or small the ALT levels do not normalize. Patients with chronic hepatitis C may have high basal level of 2'-5' AS (possibly chronic persistent infection rather than chronic active disease) and these show poorer responses to the usual IFN therapy (Solinas et al., 1993). The 2'-5'AS measurements can, therefore, give precious information to compare various IFN-α and β regimen with the aim of obtaining the best efficacy with the minimal side effects. Elevated levels of circulating ICAM-1 in the serum was found in a higher proportion of chronic hepatitis C patients unresponsive to IFN-β therapy than in those who responded well to IFN-β (Sansonno et al., 1992). This may be a reflection of the liver inflammatory process. Patients with high baseline γ-glutamyl transpeptidase also responded less to IFN-α (Battezzatti et al., 1992).

Another predictive parameter is the appearance of anti-IFN antibodies. In a 12-month treatment study with rIFN-α2a, 32% of patients developed neutralizing antibodies and only 26% of these reduced their ALT levels compared to 81% of response in the group who did not develop antibodies (Milella et al., 1993). The antibodies may also correlate with late reactivation of liver damage (Bonetti et al., 1994). Use of recombinant IFN with a structure as close as possible to the natural molecule (such as in the case of CHO produced IFN-β) may be a way to reduce antibody induction (see Section VIC). A recent report found no antibodies in hepatitis C patients treated with IFN-β (Arai et al., 1994).

Chronic Hepatitis B

IFN-α therapy of chronic hepatitis B gives 40-50% response rates with loss of viral replication which are usually sustained in 90% of

responders, unlike what is seen in hepatitis C (Perrillo and Mason, 1994; Saracco et al., 1994). Pretreatment with prednisone can improve the response rate to IFN-α, but side effects remain a problem in prolonged treatments. Despite the advent of vaccination, interest for a well tolerated IFN therapy is still intense. In a multicentre trial, IFN-β (5MIU i.m. 3 x/w for six months) was found to be efficient with optimal compliance (Capalbo et al., 1994) Satisfactory results were reported also with weekly i.v. injections of IFN-β (Kagawa et al., 1993).

An interesting aspect of Hepatitis B virus infection is that it inhibits the expression of IFN genes, in particular the IFN-β gene (Twu et al., 1988), and also the response of cells to IFN and double-stranded RNA (Foster et al., 1991). The terminal-protein (TP) region of the Hepatitis B virus genome appears to interfere with the induction of IFN-activatable genes, and patients whose liver biopsies show a large number of cells expressing the HBV terminal protein tended not to respond to IFN treatment (Foster et al., 1993). This study also showed that IFN does not reduce TP expression although it reduces expression of HBV nucleocapsid protein. In line with these effects of HBV on IFN-β synthesis and action, the 2'-5'AS levels are not elevated in patients with chronic active hepatitis B, although they are elevated in patients with chronic persistent or acute infections (Poitrine et al., 1985). However, during IFN-β or IFN-α therapy, the 2'-5'AS increases and can be used to predict the response of chronic hepatitis B patients (Nishiguchi et al., 1989).

VIII. MAJOR CLINICAL APPLICLATIONS OF IFN-β MALIGNANCIES

Several studies with IFN-β, often in combination with other anti-tumoral treatments, have indicated efficacy in prolonging survival of advanced cancer patients with solid tumors. These results, summarized in the following sections, should stimulate further clinical trials with IFN-β.

A. Non-small Cell Lung Carcinoma

Non-small cell lung carcinoma represents 75-80% of lung cancers with a world-wide incidence of about 1.8 million cases. The rationale for the use of IFN-β was based on the observation that, in addition to its antiproliferative effects, IFN-β (natural, fibroblast produced) sensitizes

a human bronchogenic carcinoma cell line to the cytotoxic action of γ-irradiation (Gould et al., 1984). Thus, A549 cells treated with 300 U/ml IFN-β for two days, then irradiated and cultured for three weeks with IFN, showed a larger decrease in colony formation than untreated cells (survival being about 0.9% vs. 3% without IFN). In this particular cell system, IFN-α did not affect radiosensitivity. IFN-β is also able to induce squamous differentiation in a human non-small cell lung carcinoma cell line (Nair et al., 1994). In a Phase I/II study of 32 patients with inoperable non-small cell lung carcinoma, mainly Stage III A/B patients with recurrent or metastatic disease, rIFN-βser was administered i.v. just prior to primary tumor irradiation (McDonald et al., 1993). Irradiation (180 cGy) was given in six courses of five days/week and the IFN-β administered for three consecutive days at the beginning of courses 1,3, and 5 at doses of 2-18 MIU (corrected units). Response to therapy measured by CT scans every three months for two years indicated an 85% response rate, with complete disappearance of tumor in 44% of patients (CR) and reduction of at least twofold in another 38% (PR). Matched historical controls (irradiated without IFN-β) would have only 15% CR and 20% PR. Survival at five years was 31% in the IFN-β and irradiated group compared to 1% survival in the matched historical group and 5% survival in the best results of conventional radiation alone (McDonald et al., 1993; Byhardt, 1993). No patient died from year 3-5 in the IFN-β group and, in the 44% of patients with CR, survival was close to 60%. If confirmed in controlled trials, IFN-β may markedly improve the prospects of radiotherapy in NSCLC without adding significant toxicity. Although IFN-α could also sensitize some malignant cells to radiation (Dritschilo et al., 1982; Namba et al., 1984), clinical studies of IFN-α with irradiation have shown only minimal responses and increased toxicity such as pneumonitis (Torrisi et al., 1986; Holsti et al., 1987; Strander and Oberg, 1992).

Another application of IFN-β has been the palliative treatment of malignant pleural effusions for which intracavitary injections of IFN-β, 5-20 MIU, was reported to be beneficiary. Response rates of 68% were recorded mainly in patients with large effusion volumes (Rosso et al., 1988).

B. Advanced Colorectal Cancer

Combination of 5-fluorouracil (5-FU) and IFN have enhanced cytotoxic effects against colorectal cancer cell lines, IFN increasing inhibi-

tion of thymidylate synthetase and 5-FU enhancing cell-mediated killing of IFN-α (Wadler and Wiernick, 1990; Neefe and John, 1991) and IFN-β (D'Atri et al., 1988). Clinical trials with combinations of IFN-α and 5FU give average response rates (mainly PR) of about 40% versus 20% for 5FU alone (see Strander and Oberg, 1992 for review). 5FU has been given i.v. at 750 mg/m2 for five days and then once weekly, the IFN-α being given s.c at 9 MIU, 3x/w. This regimen can give up to 60% PR but has some hematological and neurological toxicity (Wadler and Wiernick, 1990). Recently, a similar trial with natural IFN-β and 5FU was conducted in comparison to 5FU alone with 20 patients in each arm for one year (Villar et al., 1995). No life threatening toxicity was observed and, although the response rate was not high, a marked benefit in survival was observed in the group receiving IFN-β and 5FU: the median overall survival was 31.9 weeks for 5FU alone versus 65.9 weeks for IFN-β and 5FU ($p<0.03$), IFN-β increasing the probability of survival at one year from 34% to 72%. The mean time to progression of disease was also increased by IFN-β, from 17.7 weeks to 30.8 weeks. This trial with natural IFN-β is promising and emphasizes the need for long-term follow-up. This combination of IFN-β with 5FU may be a rather specific interesting modality since combination of IFN-β with IL-2 did not give significant effects (Barni et al., 1994).

C. Malignant Melanomas and Gliomas

IFNs have been studied alone, or in combination with IL-2 or with chemotherapy for the treatment of metastatic malignant melanoma, with promising results (Kirkwood, 1994). Post-surgery, systemic adjuvant therapy with IFN-α decreases recurrence and can increase median survival from 2.6 to 3.4 years (Borden, 1993, Cascinelli et al., 1994). In metastatic melanoma, IFN-α and IFN-β injected intralesionally have given 60% response rates but this has limited usefulness (Rosso et al., 1985; Strander and Oberg, 1992). Systemic IFN-α gives tumor response rates around 10-15%, but combinations with dacarbazine have shown up 28-38% complete responses (Garbe, 1993; Kirkwood, 1994) and up to 40% response rate was reported with IFN-α and IL-2 (Keilholz et al., 1994). Early trials of rIFN-βser in metastatic melanoma suggest it may be an alternative IFN modality (Borden et al., 1988; Schiller et al., 1988). As toxicity limits use of IL-2/IFN-α combinations (Vuoristo et al., 1994), IL-2/ IFN-β may be a better tolerated alternative (Krigel et al., 1988; De Braud et al., 1994). Since IFN-β has a stronger antiproliferative effect

than IFN-α on melanoma cells (Johns et al., 1992), which may partly contribute to the clinical effect (Garbe, 1993), further studies of IFN-β combinations on the survival of metastatic melanoma patients might be warranted. Murine models indicate that IFN-β also increases T cell infiltration of melanoma tumors (Nakayama et al., 1993) and that anti-tumoral response is not limited to the antiproliferative effect (Fleischmann et al., 1994).

In primary malignant melanoma stage I, a study of about 100 patients treated with natural IFN-β 5MIU given i.v. 3 x/w for six months suggests an effect on survival when compared to matched historical controls (Beiteke et al., 1993). A five-year survival rate of 89% was seen in the control group and a 95% survival rate was seen in the IFN-β group. Stratification of patients at higher risk (tumor thickness > 1.5mm) showed 77% survival in the control group but 95% in the IFN-β group ($p = 0.01$). At five years, 75% of the IFN-β treated were recurrence free versus 53% in the controls in this high-risk group. This may be an interesting adjuvant treatment in non-disseminated melanoma.

Glioma cells are more sensitive to IFN-β than to IFN-α (Rosenblum et al., 1990). IFN-β was applied to treatment of glioma tumors (glioblastoma and malignant astrocystomas) locally or i.v. with better results than IFN-α (Nagai and Arai, 1984; Nagai, 1988). Systemically, IFN-β alone gives response rates around 20%, similar to nitrosourea (ACNU) plus irradiation, but combination of IFN-β with ACNU and irradiation give about 40% responses (Nagai, 1991). A more recent study of 175 patients showed the highest rate of complete responses (23% CR) with i.v. IFN-β and ACNU plus irradiation (Yoshida et al., 1994). Such combination therapy with IFN-β was also effective in children with intrinsic brainstem glioma giving 27% CR and 63% PR (Wakabayashi et al., 1992). Intratumoral injections of IFN-β together with monoclonal antibodies appear promising in recurrent gliomas (Nagai, 1991) and other combinations as with suicide gene therapy may be envisaged. By i.v. injections to recurrent glioma patients, rIFN-βser alone gave 23% responses and 28% stable disease for a median of eight months (Yung et al., 1991). Negative results were also reported in both local or systemic treatments (Von Wild and Knocke, 1994). Further studies of IFN-β seem warranted, particularly to assess whether the CT scan responses which are observed in many of the above trials are true tumor regressions, a problem which has been raised in some trials with IFN-α (Strander and Oberg, 1992). Experiments in nude mice indicate that regression of previously transplanted human brain glioma tumors can indeed be achieved by intratu-

moral injection of liposome-encapsulated human IFN-β genes (Yagi et al., 1994). Mouse models suggest that alterations of the blood-brain barrier may be important for the action of exogenous IFN in gliomas (Mihara et al., 1991; Wiranowska et al., 1994).

D. Hormone-dependent Tumors: Breast Cancer

Early studies with IFN-β in patients with advanced breast cancer showed increased levels of receptors for estrogen (ER) and progesterone (PR) in biopsies of skin metastases (Pouillart et al., 1982). Since in breast tumors foci of ER-negative cells proliferate more rapidly than surrounding ER or PR positive cells (Ballare et al., 1989,1991), the increase in hormone receptors may be viewed as a differentiation effect of IFN making the growth of the cells more hormone dependent. In the human breast cancer cell line CG-5 (an MCF-7 derivative), IFN-β causes both a growth-inhibition and a 50% increase in ER and PR levels seen by hormone binding at five days (Sica et al., 1987). There may be an increased receptor synthesis since the steady state level of ER mRNA is 20% higher at 48 h after IFN-β addition (Sica et al., 1992). In ZR-75-1 cells, IFN-α increased ER expression and also made the cells more sensitive to the antiproliferative effect of the anti-estrogen Tamoxifen (TAM; Van den Berg et al., 1987). Despite variability in the ER changes seen in different cell lines, the combination of TAM and IFN appears to inhibit growth of breast cancer cells more strongly. In MCF-7 cells, IFN-β increased estrogen binding by only 10-20% but inhibited growth by up to 90%, the effect of TAM on cell growth inhibition being additive but not synergistic (Porzsolt et al., 1989). In the same study, IFN-α was less efficient than IFN-β with or without TAM. In athymic mice, the growth of estrogen-stimulated MCF-7 tumors was inhibited by IFN-β but the combination of TAM and IFN-β could totally suppress growth of xenografts, although no effect of IFN on ER levels was seen (Gibson et al., 1993).

The anti-tumor activity of TAM is probably not only due to its anti-estrogen effect at the receptor level but also to induction of growth inhibitory cytokines (Knabbe et al., 1987), so that the relation between its action and the ER level may not be a direct one. Nevertheless, in patients with advanced metastatic breast cancer TAM induces tumor regression in about 30% of total cases, the response rate of ER positive tumors being 10 times that of ER negative ones (Budzar, 1990). Since even a small modulation of ER or PR by IFN could influence the response

rate to TAM, a few clinical trials were carried out in metastatic breast cancer with IFN-β and Tamoxifen combinations. Two week courses of natural IFN-β given i.m. 3 x/w at 2 or 6 MIU increased the ER and PR levels in metastatic nodules of postmenopausal, metastatic breast cancer patients (Sica et al., 1993a). The increases were seen in about 50-60% of the 45 cases treated, without relation to the starting receptor level, confirming the variability in response of individual tumors. After IFN-β, oral TAM could be given (30 mg/day for 12 weeks) in combination with IFN-β (1 x/w) without significant toxicity, unlike what was reported for IFN-α -TAM treatment (Porzsolt et al., 1989; Macheledt et al., 1991). In 33 metastatic breast cancer patients with progressive disease during previous TAM treatment, a two-week course of IFN-β followed by a TAM-IFN-β combination produced a partial response in 24% and stable disease in 50%, whereas in 10 patients with PR or stable disease under TAM there were 3 CR with the TAM-IFN-β regimen (Buzzi et al., 1992). Additional studies indicated that in patients who had stopped responding to TAM (acquired resistance), the TAM-IFN-β combination can give around 13% objective responses and 37% disease stabilization (Buzzi et al., 1995). Association of TAM-IFN-β and retinyl palmitate gave 55% responses (24% CR, 31% PR) in metastatic breast cancer independent of receptor status (Recchia et al., 1995). A case of complete resolution of breast cancer bone metastasis following a six-month TAM-IFN-β treatment was reported (Campisi et al., 1993) but the role of IFNs in advanced breast cancer management is still controversial (Repetto et al., 1993). Whether or not the improvement in TAM response by IFN-β is essentially due to an effect on ER levels remains unclear but larger controlled trials appear warranted to evaluate the well tolerated TAM-IFN-β combination in advanced metastatic breast cancer patients.

Modulation of hormone-therapy by IFN-β may be applicable to other malignancies. *In vitro*, incubation of endometrial cancer tissue fragments with IFN-β resulted in an increase of cytosolic ER and PR (DeCicco et al., 1988), and administration of 2-6 MIU IFN-β i.m. three times in one week to primary endometrial carcinoma patients increased the tumor ER and PR levels in more than 50% of cases (Sica et al., 1993b). Androgen receptors could be up-regulated by IFN-β in the human prostatic PC-3 cell line, with increase of the antiproliferative action of the antiandrogen hydroxyflutamide (Sica et al., 1994). A combination of TAM-IFN-β was tested in unresectable liver cancer with results suggesting it could be an effective alternative to arterial chemoembolization (Maffei et al., 1994)

whereas IFN-β given in other combinations was ineffective in such cancer patients (Colleoni et al., 1993).

E. Prospects in Other Viral and Malignant Diseases

Hairy cell leukemia, the first human disease found to be highly responsive to IFN-α therapy, can be successfully treated also with natural IFN-β given i.v. (Liberati et al., 1990b) or with rIFN-βser (Glaspy et al., 1989; Wiernick et al., 1990). Treatment with natural IFN-β is effective also i.m. and even in patients resistant to IFN-α as shown *in vivo* and *in vitro* (Michalevitz et al., 1988). However, in the major hematological indication of IFN-α today, chronic myeloid leukemia (CML), IFN-β has not been clinically effective in the chronic phase despite its *in vitro* effect on CML progenitor growth (Aulitzky et al., 1993). In this leukemia and lymphomas such as multiple myeloma, IFN-α appears more active than IFN-β, at least when IFN-β is given by i.v. In other indications of IFN-α, in solid tumors such as neuroendocrine gut carcinoids (Strander and Oberg, 1992), there are indications that IFN-β treatment may replace IFN-α because of its better tolerance in prolonged treatments. The low incidence of hematological side-effects associated with IFN-β therapy may be of interest also in treatment of AIDS-related Kaposi sarcoma (Miles et al., 1990) in particular when azidothymidine (AZT) is also administered (Brockmeyer et al., 1989). IFN-β synergizes with AZT to inhibit HIV (Willams and Colby, 1989), may increase its availability *in vivo* and protect against AZT-induced chromosomal breaks (Nokta et al., 1991; Shafik et al., 1991). Interferons α,β have multiple effects on HIV replication (Pitha, 1991; Coccia et al., 1994) and IFN-β has both anti-viral and immunological effects also on HTLV-1 infections (D'onofrio et al., 1988; Fuggetta et al., 1990). New strategies to block retroviral infection and enhance resistance of target lymphocytes to HIV by IFN-β autocrine secretion are being investigated through gene therapy approaches (Veillard et al., 1994). Finally, early treatment of asymptomatic AIDS patients with IFN-β (Oka et al., 1989; Borucki et al., 1990) are of interest because the better tolerance of IFN-β could allow prolonged treatments and less toxic combinations with other drugs.

REFERENCES

Abdollahi, A., Lord, K.A., Hoffman-Lieberman, B., & Liebermann, D.A. (1991) Interferon Regulatory Factor 1 is a myeloid differentiation primary response gene

induced by Interleukin-6 and Leukemia Inhibitory Factor: Role in growth inhibition. Cell Growth Differ. 2, 401-407.

Aboagye-Mathiesen, G., Toth, F.D., Hager, H., Zdravkovic, M., Petersen, P.M., Villadsen, J.A., Zachar, V., & Ebbesen, P. (1993). Human trophoblast interferons. Antiviral Res. 22, 91-105.

Abramovich, C., Chebath, J., & Revel, M. (1994a). The human Interferon α-Receptor protein confers differential responses to human Interferon-β versus Interferon-α subtypes in mouse and hamster cell transfectants. Cytokine 6, 414-424.

Abramovich, C., Shulman, L.M., Ratovitski, E., Harroch, S., Tovey, M., Eid, P., & Revel, M. (1994b). Differential tyrosine phosphorylation of the IFNAR chain of the type I Interferon Receptor and of an associated surface protein in response to IFN-α and IFN-β. EMBO J. 13, 5871-5877.

Ambramovich, C., Yakobson, B., Cebath, J., & Revel, M. (1996). A protein-arginine methyltransferase binds to the intracytoplasmic domain of the IFNAR1 chain in the type I Interferon receptor. EMBO J. 15.

Adams, F., Quesada, J.R., & Gutterman, J.U. (1984). Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. J. Am. Med. Assn. 252, 938-941.

Arai, K., Shindo, M., & Okuno, T. (1994). Anti-Interferon antibody in chronic hepatitis C. Nippon Rinsho 52, 1929-1934.

Arima, T., Shimomura, H., & Nakagawa, J.. (1986) Treatment of non-A non-B hepatitis with human fibroblast Interferon. Hepatol. 6, 117a.

Au, W.C., Raj, N.K.B., Pine, R., & Pitha, P.M. (1992). Distinct activation of murine Interferon-α promoter region by IRF-1/ISGF-2 and virus infection. Nucleic Acids Res. 20, 2877-2884.

Aulitzky, W.E., Peschel, C., Despres, D., Aman, J., Trautman, P., Tilg, H., Rudolf, G., Huttmann, H., Obermeier, J., Herold, M., et al. (1993). Divergent *in vivo* and *in vitro* antileukemic activity of recombinant Interferon beta in patients with chronic-phase chronic myelogenous leukemia. Ann. Hematol. 67, 205-211.

Ballare, C., Bravo, A.I., Laucella, S., Sorin, I., Cerdeiro, R., Loza, J., Sousa Martinez, F., Guman, N., & Mordoh, J. (1989). DNA synthesis in estrogen receptor-positive human breast cancer takes place preferentially in estrogen receptor-negative cells. Cancer 64, 842-848.

Ballare, C., Bravo, A.U., Sorin, I., Guman, N., Schiaffi, J.A., Yomha, R., Bagnati, A., Lema, B., & Mordoh, J. (1991). The expression of progesterone receptors coincides with an arrest of DNA synthesis in human breast cancer. Cancer 67, 1352-1358.

Barna, B.P., Chou, S.M., Jacobs, B., Yen-Lieberman, B., & Ransohoff, R.M. (1989). Interferon-β impairs induction of HLA-DR antigen expression in cultured human astrocytes. J. Neuroimmunol. 20, 39-44.

Barni, S., Lissoni, P., Ardizzola, A., Vigore, L., Vezzo, R., Rescaldani, R., & Tancini, G. (1994). Immunotherapy with low-dose subcutaneous interleukin-2 plus beta-interferon as a second line therapy for metastatic colorectal carcinoma. Tumori 79, 343-346.

Basham, T.Y., & Merigan, T.C.(1983). Recombinant interferon-gamma increases HLA-DR synthesis and expression. J. Immunol. 130, 1492-1494.

Batcheler, L.M., Bonham, D.G., Collins, R., & Liddell, H.S. (1986). Topical Interferon cream for the treatment of herpes genitalis: A double-blind controlled trial. Aust. NZ J. Obstet. Gynaecol. 26, 239-241.

Battezzati, P.M., Podda, M., Bruno, S., Zuin, M., Battezzati, P.M., Podda, M., Bruno, S., Zuin, M., Crosignani, A., Camisasca, M., Chiesa, A., Petroni, M.L., Russo, A., Gallotti, P., et al. (1992). Factors predicting early response to treatment with recombinant interferon-alpha2a in chronic non-A, non-B hepatitis. Preliminary report of a long-term trial. Ital. J. Gastroenterol. 24, 481-484.

Bazan, J.F. (1990). Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA 87, 6934-6938.

Beiteke, U., Ruppert, P., Garbe, C., Oxenfarth, R., Kastl, I., Turker, T., Tronnier, H., & Frosch, P.J. (1993). Adjuvant therapy of primary malignant melanoma with human interferon-beta. Significant survival advantage in 96 treated patients in comparison to 288 untreated sympromatic controls. Hautarzt 44, 365-371.

Belhumeur, P., Lanoix, J., Blais, Y., Forget, D., Steyaert, A., & Skup, D. (1993). Action of spontaneously produced beta-Interferon in differentiation of embryonal carcinoma cells through an autoinduction mechanism. Mol. Cell. Biol. 13, 2846-2857.

Ben-Simchon, L.(1995). Transcription factors binding to IFN-stimulatable responsive enhancers (ISRE): Structural analysis and regulation during cell cycle and differentiation. Ph.D. Thesis. Weizmann Institute of Science, Rehovot 76100, Israel.

Benech, P., Vigneron, M., Peretz, D., Revel, M., & Chebath, J. (1987). Interferon-responsive regulatory elements in the promoter of the human 2'-5' oligo A synthetase gene. Mol. Cell. Biol. 7, 4498-4504.

Benoit, P., Maguire, D., Plavec, I., Kocher, H., Tovey, M., & Mayer, F. (1993). A monoclonal antibody to recombinant human IFN-α receptor inhibits biologic activity of several species of human IFN-α ,IFN-β and IFN-ω. Detection of heterogeneity of the cellular type I IFN receptor. J. Immunol. 150, 707-716.

Bever, C.T., & Whitaker, J.N. (1985). Proteinases in inflammatory demyelinating disease. Springer Semin. Immunopathol. 8, 235-250.

Bhuiya, T.A., Shodell, M., Fitzgerald-Bocarsly, P.A., Murasko, D., Shah, K., Drake, D., & Siegal, F.P. (1994). Interferon-α generation in mice responding to challenge with UV-inactivated Herpes Simplex Virus. J. Interferon Res. 14, 17-24.

Bickel, M., Dveksler, G., Dieffenbacher, C.W., Ruhl, S., Midura, S.B., & Pluznick, D.H. (1990). Induction of Interferon-β and 2'-5' oligoadenylate synthetase mRNAs during differentiation of murine myeloid cells. Cytokine 2, 238-246.

Billiau, A. (1981). Interferon therapy: Pharmacokinetics and pharmacological aspects. Arch. Virol. 67, 121-133.

Birnbaum, M., Trink, B., Shainberg, A., & Salzberg, S. (1990). Activation of the Interferon system during myogenesis *in vitro*. Differentiation 45, 138-145.

Bocci, V. (1992). Pharmacokinetics of Interferons and routes of administration. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., Fleischmann, W.R., Hughes, T.K., Jr., Klimpel, G.R., Niesel, D.W., Stanton, G.J., & Tyring, S.K., Eds.). The University of Texas Medical Branch at Galveston, pp. 417-425.

Bocci, V., Pacini, A., Bandinelli, L., Pessina, G.P., Muscetolla, M., & Paulesu, L. (1982). The role of liver in the catabolism of human-α and β Interferon. J. Gen. Virol. 60, 397-400.

Bocci, V., Pessina, G.P., Paulesu, L., Muscettola, M., & Valeri, A. (1988). The lymphatic route. V. Distribution of natural Interferon-beta in rabbit plasma and lymph. J. Interferon Res. 8, 633-640.

Bonetti, P., Diodati, G., Drago, C., Casarin, C., Scaccabarozzi, S., Realdi, G., Ruol, A., & Alberti, A. (1994). Interferon antibodies in patients with chronic hepatitis C virus infection treated with recombinant Interferon alpha-2a. J. Hepatol. 20, 416-420.

Borden, E.C. (1988). Augmented tumor-associated antigen expression by Interferons. J. Natl. Cancer Inst. 80, 148-149.

Borden, E.C. (1993). Integration into combined modality therapies. J. Interferon Res. 13, Suppl 1, S188

Borden, E.C., Hogan, T.F., & Voelkel, J.G. (1982). The comparative antiproliferative activity *in vitro* of natural Interferons α and β for diploid and transformed human cells. Cancer Res. 42, 4948-4953.

Borden, E.C., Hawkins, M.J., Sielaff, K.M., Storer, B.M., Schiesel, J.D., & Smalley, R.V. (1988). Clinical and biological effects of recombinant Interferon-β administered intravenously daily in Phase I trial. J. Interferon Res. 8, 357-366.

Borden, E.C., Rinehart, J.J., Storer, B.E., Trump, D.L., Paulnock, D.M., & Teitelbaum, A.P. (1990). Biological and clinical effects of Interferon-βser at two doses. J. Interferon Res. 10, 559-570.

Bornemann, L.D., Spiegel, H.E., Dziewanowska, Z.E., Krown, S.E., & Colburn, W.A. (1985). Intravenous and intramuscular pharmacokinetics of recombinant leukocyte A Interferon. Eur. J. Clin. Pharmacol. 28, 469-476.

Bornstein, J., Ben-David, Y., Atad, J., Pascal, B., Revel, M., & Abramovici, H. (1993a). Treatment of cervical intrepithelial neoplasia and invasive squamous cell carcinoma by interferon. Obstet. Gynecol. Survey 48, 251-260.

Bornstein, J., Pascal, B., & Abramovici, H. (1993b). Intramuscular β-Interferon treatment for severe vulvar vestibulitis. J. Reprod. Med. 38, 117-120.

Borucki, M.J., VonRoenn, J.H., Williams, R.N., & Pollard, R.B. (1990). A multicenter open-label study of subcutaneously administered recombinant Interferon-beta in patients at risk for progression to AIDS. Int. Conf. AIDS 6, 201.

Branca, A.A., & Baglioni, C. (1981). Evidence that type I and II Interferons have different receptors. Nature 294, 768-770.

Brand, C.M., Leadbeater, L., Bellati, G., Marotta, F., & Ideo, G. (1993). Antibodies developing against a single recombinant Interferon protein may neutralize many other Interferon-α subtypes. J. Interferon Res. 13, 121-125.

Brockmeyer, N.H., Mertins, L., Maleba, R., Binick, R. & Goos, M. (1989). Regression of Kaposi's sarcoma and improvement of performance status by a combined Interferon-beta and zidovudine therapy in AIDS patients. J. Invest. Dermatol. 93, 544.

Brod, S.A., & Burns, D.K. (1994). Suppression of relapsing experimental autoimmune encephalomyelitis in the SJL/J mouse by oral administration of type I interferons. Neurology 44, 1144-1148.

Brunelleschi, S., Fallani, S., Novelli, A., Reali, E.F., Rubino, A., Spezia, R., Tarli, S., Vanni, L., Dianzani, C., Viano, I., & Fantozzi, R. (1988). Modulation of neutrophil functions by a beta-Interferon of human origin. J. Biol. Regul. Homeost. Agents 2, 93-98.

Budzar, A.U. (1990). Current status of endocrine treatment of carcinoma of the breast. Sem. Surg. Oncol. 6, 77-82.

Buzzi, E., Brugia, M., Rossi, G., Giustini, L., Scoponi, C., & Sica, G. (1992). Combination of beta-Interferon and Tamoxifen as a new way to overcome clinical resistance to Tamoxifen in advanced breast cancer. Anticancer Res. 12, 869-872.

Buzzi, E., Brugia, M., Trippa, F., Rossi, G., Trivisonne, R., Giustini, L., Scoponi, C., Pinaglia D., Capparella, V., & Sica. G, (1995). Natural Interferon-beta and tamoxifen in hormone-resistant patients with advanced breast cancer. Anticancer Res. 15, 2187-2190.

Byhardt, R.W. (1993). Can Interferon beta make the radiation therapy team for treatment of Non-small Cell Lung Cancer? Int. J. Radiation Oncology Biol. Phys. 27, 753-756.

Campisi, C., Terenzi, S., Bandierini., A, Ferrone, L., Mozzicafreddo, A., Rafaele M., Zappala, A., & Tomao, S. (1993). Complete resolution of breast cancer bone metastasis through the use of beta-interferon and tamoxifen. Eur. J. Gynaecol. Oncol. 14, 479-483.

Capalbo, M., Palmisani, L., Bonino, F., Pellas, C., & Maset, J. (1994). Intramuscular natural beta-interferon in the treatment of chronic hepatitis B: A multicenter trial. Ital. J. Gastroenterol. 26, 238-241.

Carlin, J.M., Borden, E.C., & Byrne, G.I. (1989). Interferon-induced indoleamine 2,3-dioxygenase activity inhibits *Chlamidia psittaci* replication in human macrophages. J. Interferon Res. 9, 329-337.

Carreno, V., Marriott, E., & Quiroga, J.A. (1993). Other approaches to the treatment of chronic viral hepatitis. J. Hepatol. 17, S127-129.

Cascinelli, N., Bufalino, R. Morabito, A., & Mackie, R. (1994). Results of adjuvant interferon study in WHO melanoma programme. Lancet 343, 913-914.

Cavallieri, R.L., Havell, E.A., Vilcek, J., & Pestka, S. (1977). Induction and decay of human fibroblast Interferon mRNA. Proc. Natl. Acad. Sci. USA 74, 4415-4419.

Chebath, J., Benech, P., Revel, M., & Vigneron, M. (1987). Constitutive expression of 2'-5' oligoadenylate synthetase confers resistance to picornavirus infection. Nature 330, 587-588.

Chebath, J., & Revel, M. (1992). The 2-5A system: 2-5 A synthetases, isospecies and functions. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., Fleischmann, R., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Stanton, G., & Tyring, S.K. Eds.). The University of Texas Medical Branch at Galveston, Galveston, TX, pp. 225-236.

Chemello, L., Bernardinello, E., Silvestri, E., Cavaletto, L., Pontisso, P., & Alberti, A. (1995). Pilot study on the efficacy of natural interferon-beta therapy in Italian patients with chronic hepatitis C. Int. Hepatol. Commun. In press.

Cheng, J.Q., Jhanwar, S.C., Lu, Y.Y., & Testa, J.R. (1993). Homozygous deletions within 9p21-p22 identify a small critical region of chromosomal loss in human malignant mesotheliomas. Cancer Res. 53, 4761-4763.

Chernajovsky, Y., Mory, Y., Chen, L., Marks, Z., Novick, D., Rubinstein, M., & Revel, M. (1984). Efficient constitutive production of human fibroblast Interferon by hamster cells transformed with the IFN-β1 gene fused to an SV40 early promoter. DNA 3, 297-308.

Chiang, J., Gloff, C.A., Soike, K.F., & Williams, G. (1993). Pharmacokinetics of recombinant human Interferon-BetaSer in healthy volunteers and its effect on serum neopterin. Pharm. Res. 10, 567-572.

Chofflon, M., Juillard, C., Juillard, P., Gauthier, G., & Grau, G.E. (1992). Tumor necrosis factor-α production as a possible predictor of relapse in patients with multiple sclerosis. Eur. Cyt. Netw. 3, 523-531.

Chong, K.L., Feng, L., Schappert, K., Meurs, E., Donahue, T.F., Friesen, J.D., Hovanessian, A.G., & Williams, B.R. (1992.) Human p68 kinase exhibits growth suppression in yeast and homology to the translational regulator GCN2. EMBO J. 11, 1553-1562.

Cleary, C.M., Donnelly, R.J., Soh, J,, Marianom T.M., & Pestka, S. (1994). Knockout and reconstitution of a functional human type I receptor complex. J. Biol. Chem. 269, 18747-18749.

Coccia, E.M., Krust, B., & Hovanessian, A.G. (1994). Specific inhibition of viral protein synthesis in HIV-infected cells in response to Interferon treatment. J. Biol. Chem. 269, 23087-23094.

Cohen, A., Hannigan, G.E., Williams, B.R.G., & Lingwood, C.A. (1987). Roles of globotriosyl- galabiosylceramide in verotoxin binding and high affinity Interferon receptors. J. Biol. Chem. 262, 17088-17091.

Cohen, B., Gothelf, Y., Vaiman, D., Chen, L., Revel, M., & Chebath, J. (1991). Interfleukin-6 induces the 2'-5' oligoadenylate synthetase gene in M1 cells through an effect on the Interferon-responsive enhancer. Cytokine 3, 83-91.

Cohen, L., & Hiscott, J. (1992). Characterization of TH3, an induction-specific protein interacting with the Interferon beta promoter. Virology 191, 589-599.

Colamonici, O.R., & Domanski, P. (1993). Identification of a novel subunit of the type I Interferon Receptor localized to chromosome 21. J. Biol. Chem. 268, 10895-10899.

Colamonici, O.R., Pfeffer, L.M., D'Alessandro, F, R., Cruciani, R.A., & Diaz, M.O. (1992). Multichain structure of the IFN-α receptor on hematopoietic cells. J. Immunol. 148, 2126-2132.

Colamonici, O.R., Yan, H., Domanski, P., Handa, R., Smalley, D., Mullersman, J., Witte, M., Krishnan, K., & Krolevski, J. (1994). Direct binding and tyrosine phosphorylation of the α-subunit of the type I IFN receptor by the p135TYK2 tyrosine kinase. Mol. Cell. Biol. 14, 8133-8152.

Colby, C.B., Inoue, M., Thompson, M., & Tan, Y.H. (1984). Immunological differentiation between *E.coli* and CHO cell-derived recombinant and natural human beta-interferons. J. Immunol. 133, 3091-3095.

Coleman, A., Fountain, J.W., Nobori, T., Olopade, O.I., Robertson, G., Housman, D.E., & Lugo, T.G. (1994). Distinct deletions of chromsome 9p associated with melanoma versus glioma, lung cancer and leukemia. Cancer Res. 54, 344-348.

Colleoni, M., Bussoni, R., Bajetta E., Bochicchio, A.M., Bartoli, C., Audisio, R., Bonfanti, G., & Nole, F. (1993). A phase II study of mitotranxone combined with beta-Interferon in unresectable hepatocellular carcinoma. Cancer 72, 3196-3201.

Condylomata International Collaborative Study Group. (1993). Recurrent condylomata acuminata treated with recombinant interferon alpha-2a. A multicenter double-blind placebo-controlled clinical trial. Acta Derm. Venereol. 73, 223-226.

Conrad, H.S., Egge, H., Katalinic, J.P., Reiser, W., Siklosi, T., & Schaper, K. (1987). Structure of the carbohydrate moiety of human Interferon-β secreted by a recombinant Chinese hamster ovary cell line. J. Biol. Chem. 262, 14600-14605.

Content, J., De Wit, L., Tavernier, J., & Fiers, W. (1983). Human fibroblast Interferon RNA transcripts of different sizes in poly rI:rC induced cells. Nucleic Acids Res. 11, 2627-2638.

Corey, L., & Spear, P.C. (1986). Infections with herpes simplex viruses. N. Eng. J. Med. 314, 686-691.

Costa, S., Poggi, M.G., Palmisano, L., Syrjanen, S., Yliskosky, M., & Syrjanen, K.J. (1988). Intramuscular β-interferon treatment of human papillomavirus lesions in the lower female genital tract. The Cervix 6, 203-212.

D'Atri, S., Fuggetta, M.P., Giganti, G., Tentori, L., Roselli, M., Nunziata, C., Pastore, S., Bonmassar E., & DeVecchis, L. (1988). Comparative studies between *in vitro* and *in vivo* effects of beta-Interferon on natural killer activity and its relevance to immunochemotherapy. Cancer Immunol. Immunother. 27, 163-170.

Daigneault, L., Haggarty, A., Meng, Q.H., & Skup, D. (1992). Two distinct pathways of Interferon induction as revealed by 2-amino purine. Nucleic Acids Res. 20, 2749-2754.

Darnell, J.E. Kerr, I.M., & Stark, G.R. (1994). Jak-Stat pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

David, M., Petricoin, E., III, Benjamin, C., Pine, R., Weber, M.J., & Larner, A.C. (1995). Requirement for MAP kinase (ERK2) activity in Interferon α- and Interferon β-stimulated gene expression through Stat proteins. Science. 269, 1721-1723.

Davis, G.L. (1990). Recombinant alfa-interferon treatment of non-A, non-B (type C) hepatitis: Review of studies and recommendations for treatment. J. Hepatol. 11, S72-S77.

De Aloysio, D., Miliffi, L., Iannicelli, T., Penacchioni, P., & Bottiglioni, F. (1994). Intramuscular interferon-beta treatment of cervical intraepithelial neoplasia II associated with human papillomavirus infection. Acta Obstet. Gynecol. Scand. 73, 420-424,

De Cicco, F., Sica, G., Benedetto, M.T., Ciabattoni, G., Rossiello, F., Nicosia, A., Lupi, G., Iacopino, F., Mancuso, S., & Dell'Acqua, S. (1988). *In vitro* effects of β-Interferon on steroid receptors and prostanglandin output in human endomerial adenocarcinoma. J. Steroid Biochem. 30, 359-362.

De Braud, F., Colleoni, M., Noli, F., & Bajetta, E. (1994). Home treatment with interleukin-2 and beta-interferon in patients with colorectal cancer and hepatocellular carcinoma. Oncology 51, 472-476.

De Vos, A.M., Ultsch, M., & Kossiakoff, A.A. (1992). Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. Science 255, 306-312.

Decker, T. (1992). Double-stranded RNA and Interferon-α induce transcription through different molecular mechanisms. J. Interferon Res. 12, 445-448.

DePalo, G., Stefanon, B., & Merola, M. (1990). Long-term results of intralesional human fibroblast interferon in papillomavirus-associated CIN or VIN. J. Exp. Clin. Canc. Res. 12, S FC/174, 1990.

Derynck, R., Remaut, E., Saman, E., Stanssens, P., DeClercq, J., Content, J., & Fiers, W. (1980). Expression of human fibroblast Interferon gene in *Escherichia coli.* Nature 287, 193-197.

Devajyothi, C., Kalvakolanu, I., Babcock, G.T., Vasavada, H.A., Howe, P.H., & Ransohoff, R.M. (1993). Inhibition of Interferon-γ induced Major Histocompatibility complex class II gene transcription by Interferon-β and type β1 Transforming growth factor in human astrocytoma cells: Definition of cis-element. J. Biol. Chem. 268, 18794-18800.

Dhib-Jalbut, S.S., & Cowan, E.P. (1993). Direct evidence that Interferon-beta mediates enhanced HLA-class I expression in measles virus-infected cells. J. Immunol. 151, 6248-6258.

Dianzani, F. (1992). Interferon treatments: How to use an endogenous system as a therapeutic agent. J. Interferon Res. 13, special issue, May, 109-118.

Diaz, M.O., Ziemin, S., Lebeau, M.M., Pitha, P.M., Smith, S.D., Chilcote, R.R., & Rowley, J.D. (1988). Homozygous deletion of the alpha- and beta-1-Interferon genes in human leukemia and derived cell lines. Proc. Natl. Acad. Sci. USA 85, 5259-5263.

Diaz, M.O., Pomykala, H.M., Bohlander, S.K., Maltepe, E., Malik, K., Brownstein, B., & Olopade, O.I. (1994). Structure of human Type-I Interferon gene cluster determined from a YAC clone contig. Genomics 22, 540-552.

DiMarco, S., Grutter, M.G., Priestle, J.P., & Horisberger, M.A. (1994). Mutational analysis of the structure-function relationship in Interferon-alpha. Biochem. Biophys. Res. Commun. 202, 1445-1451.

Dinter, H., & Hauser, H. (1987). Cooperative interaction of multiple DNA elements in the human Interferon-β promoter. Eur. J. Biochem. 166, 103-109.

Dolei, A., Ameflio, F., Capobianchi, M.R., & Tosi, R. (1982). Human beta type Interferon enhances the expression and shedding of IA-like antigens,, comparison to HLA-A,B,C and beta-2 microglobulin. Antiviral Res. 1, 367-382.

Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., & Colamonici, O.R. (1995). Cloning and expression of a long form of the beta subunit of the Interferon alpha beta receptor that is required for signaling. J. Biol. Chem. 270, 21606-21611.

D'Onofrio, C., Perno, C.F., Mazzetti, P., Graziani, G., Calio, R., & Bonmassar, E. (1988). Depression of early phase HTLV-1 infection *in vitro* mediated by human beta-Interferon. Br. J. Cancer 57, 481-488.

Dritschilo, A., Mossman, K., Gray, M., & Sreevalsan, T. (1982). Potentiation of radiation injury by interferon. Am. J. Clin. Oncol. 5, 79-82.

Du, W., & Maniatis, T. (1992). An ATF/CREB element is required for virus induction of the human Interferon-β gene. Proc. Natl. Acad. Sci. USA 89, 2150-2154.

Du, W., Thanos, D., & Maniatis, T. (1993). Mechanism of transcriptional synergism between distinct virus-inducible enhancer elements. Cell 74, 887-898.

Dummer, R., Muller, W., Nestle, F., Wiede, J., Dues, J., Lechner, W., Haubitz, I., Wolf, W., Bill, E., & Burg, G. (1991). Formation of neutralizing antibodies against natural Interferon-β but not against recombinant Interferon-γ during adjuvant therapy for high risk malignant melanoma patients. Cancer 67, 2300-2304.

Durelli, L., Bongiovanni, M.R., Cavallo, R., Ferrero, B., Ferri, R., Ferrio, M.F., Bzadac, G.B., Riva, A., Vai, S., Geuna, M., et al. (1994). Chronic systemic high-dose

recombinant interferon alfa-2a reduces exacerbation rate, MRI signs of disease activity and lymphocyte interferon gamma production in relapsing-remitting Multiple Sclerosis. Neurology 44, 406-413.

Edge, M.D., Camble, R., Moore, V.E., Hockeny, R.C., Carr, F.C., & Filton, J.E. (1986). Interferon analogues from synthetic genes: An approach to protein structure-activity studies. In: *Interferon 7* (Gresser, I., Ed.). Academic Press, New York, pp. 1-46,

Enoch, T., Zinn, K., & Maniatis, T. (1986). Activation of the human β-Interferon gene requires an Interferon-inducible factor. Mol. Cell. Biol. 6, 801-810.

Fabra, A., Nakajima, M., Bucana, C.D., & Fidler, I.J. (1992). Modulation of the invasive phenotype of human colon carcinoma cells by organ specific fibroblasts of nude mice. Differentiation 52, 101-110.

Fan, C.M. & Maniatis, T. (1989). Two different virus-inducible elements are required for human β-Interferon gene regulation. EMBO J. 8, 101-110.

Fan, C.M., & Maniatis, T.(1990).A DNA-binding protein containing two widely separated zinc finger motifs that recognize the same DNA sequence. Genes & Dev. 4, 29-35.

Fernandez, O., Antiquedad, A., Aribisu, T., Capdevila, A., de Castro, P., Correa de Sa, J.C., Garcia-Merino, J.A., Izquierdo, G., Magalhaes, A., Montalban, X., & Zarranz, J.J. (1995). Treatment of relapsing-remitting multiple sclerosis with natural Interferon beta: A multicenter, randomized clinical trial. Multiple Sclerosis 1, S67-S69.

Fierlbeck, G., Schreiner, T., Schaber, B., Walser, A., & Rassner, G. (1994). Neutralizing Interferon-β antibodies in melanoma patients treated with recombinant and natural Interferon-β. Cancer Immunol. Immunother. 39, 263-268.

Fish, E.N. (1992). Definition of receptor binding domains in Interferon-α. J. Interferon Res. 12, 257-266.

Fleischmann, C.M., Stanton, G.J., & Fleishmann, W.R. (1994). Enhanced *in vivo* sensitivity to interferon with *in vitro* resistant B16 tumor cells in mice. Cancer Immunol. Immunother. 39, 148-154.

Foster, G.R., Ackrill, A.M., Goldin, R.D., Kerr, I.M., Thomas, H.C., & Stark, G.R. (1991). Expression of the terminal protein region of hepatitis B virus inhibits cellular responses to interferons α and γ and double-stranded RNA. Proc. Natl. Acad. Sci. USA 88, 2888-2892.

Foster, G.R., Goldin, R.D., Hay, A., McGarvey, M.J., Stark, G.R., & Thomas, H.C. (1993). Expression of the terminal protein of hepatitis B virus associated with failure to respond to interferon therapy. Hepatology 17, 757-762.

Fu, X.Y., Schindler, C., Improta, T., Aebersold, R., & Darnell, J.E. (1992). The proteins of ISGF3, the interferon-α induced transcriptional activator define a gene family involved in signal transduction. Proc. Natl. Acad. Sci. USA 89, 7840-7843.

Fuggetta, M.P., D'Onofrio, C., & Bonmassar, E. (1990). Role of biological response modifiers in immunochemotherapy of solid tumors and retroviral induced leukemia. Ann. Ist. Super. Sanita 26, 385-395.

Fujihara, M., Ito, N., Pace, J.L., Watanabe, Y., Russell, S.W., & Suzuki, T. (1994). Role of endogenous Interferon-beta in lipopolysaccharide-triggered activation of the inducible nitric-oxide synthase gene in a mouse macrophage cell line. J. Biol. Chem. 269, 12773-12778.

Fujita, T., & Kohno, S. (1981). Studies on Interferon priming: Cellular response to viral and nonviral inducers and requirement of protein synthesis. Virology 112, 62-69.

Fujita, T., Shibuya, H., Hotta, H., Yamanishi, K., & Taniguchi, T. (1987). Interferon-β gene regulation: Tandemly repeated sequences of a synthetic 6 bp oligomer function as a virus-inducible enhancer. Cell 49, 357-367.

Fujita, T., Sakakibara, J., Sudo, Y., Miyamoto, M., Kimura, Y., & Taniguchi, T. (1988). Evidence for a nuclear factor(s), IRF-1, mediating induction and silencing properties to human IFN-β gene regulatory elements. EMBO J. 7, 3397-3405.

Fujita, T., Kimura, Y., Miyamoto, M., Barsoumian, E.L., & Taniguchi, T. (1989a). Induction of endogenous IFN-α and IFN-β genes by a regulatory transcription factor IRF-1. Nature 337, 270-272.

Fujita, T., Miyamoto, M., Kimura, Y., Hammer, J., & Taniguchi, T. (1989b). Involvement of a cis-element that binds an H2TF-1/NF-κB like factor(s) in virus-induced Interferon-β gene expression. Nucleic Acids Res. 17, 3335-3346.

Fujita, T., Reis, L., Watanabe, N., Kimura, Y., Taniguchi, T., & Vilcek, J. (1989c). Induction of the transcription factor IRF-1 and Interferon-β mRNAs by cytokines and activators of second messenger pathways. Proc. Natl. Acad. Sci. USA 86, 9936-9940.

Fukui, S., Tohyama, H., & Iwabushi, S. (1994). Interferon therapy for acute hepatitis C: Changes in serum markers associated with HCV and clinical effects. Nippon Rinsho 52, 1847-1851.

Garbe, C. (1993). Chemotherapy and chemoimmunotherapy in disseminated malignant melanoma. Melanoma Res. 3, 291-299.

Garoufalis, E., Kwan, I., Lin, R., Mustafa, A., Pepin, N., Roulston, A. Lacoste, J., & Hiscott, J. (1994). Viral induction of the human beta Interferon promoter: Modulation of transcription by NF-kappa B/rel proteins and Interferon Regulatory Factors. J. Virol. 68, 4707-4715.

Garzetti, G.G., Ciavattini, A., Romanini, C., Goteri, G., Tranquilli, A.L., Muzziolo, M., & Fabris, N. (1994). Interferon alpha-2b treatment of cervical intraepithelial neoplasia grade 2: Modulation of natural killer cell. Gynecol. Obstet. Invets. 37, 204-209.

Gazitt, Y., Ben-Bassat, H., Wallach, D., Revel, M., & Schattner, A. (1984). Interferon-induced cytotoxicity and (2'-5') oligo A synthetase activity in T cells. Differences in responsiveness among T cells from individuals and among lymphoblastoid T-cell lines. Clin. Immunol. Immunopathol. 30, 71-79.

Ghersetich, I., & Lotti, T. (1994). Alpha-Interferon cream restores decreased levels of Langerhans/indeterminate (CD1a+) cells in aged and PUVA-treated skin. Skin Pharmacol. 7, 118-120.

Ghislain, J., Lingwood, C.A., & Fish. E.N. (1994). Evidence for glycosphingolipid modification of the type I Interferon Receptor. J. Immunol. 153, 3655-3663.

Giacomini, P., Fraioli, R., Nistico, P., Tecce, R., Nicotra, M.R., DiFilippo, F., Fischer, P.B., & Natali, P.G. (1990). Modulation of the antigenic phenotype of early passage human melanoma cells derived from multiple autologous metastases by recombinant human leukocyte, fibroblast and immune Interferon. Int. J. Cancer 46, 539-546.

Giannelli, G., Antonelli. G., Fera, G., Dianzani, F., & Schiraldi, O. (1993). 2'-5' oligoadenylate synthetase activity as a responsive marker during IFN therapy for chronic hepatitis C. J. Interferon Res. 13, 57-60.

Gibson, D.F., Johnson, D.A., Goldstein, D., Langan-Fahey, S.M., Borden, E.C., & Jordan, V.C. (1993). Human recombinant Interferon-Beta$_{ser}$ and Tamoxifen: Growth supressive effects for the human breast carcinoma MCF-7 grown in the athymic mouse. Breast Cancer Res. Treat. 25, 141-150.

Glaspy, J.A., Marcus, S.G., Ambersley, J., & Golde, D.W. (1989). Recombinant beta-serine Interferon in hairy cell leukemia compared prospectively with results with recombinant alpha-Interferon. Cancer 64, 409-413.

Glezerman, M., Lunenfeld, E., Cohen, V., Sarov, I., Movshovitz, M., Doerner, T., Shoham, J., & Revel, M. (1988a). Placebo-controlled trial of topical Interferon in labial and genital herpes. Lancet 1, 150-152.

Glezerman, M., Movshovitz, M., Doerner, T., Shoham, J., & Revel, M. (1988b). Topical Interferon-beta reduces frequency of recurrences in labial and genital herpes: Double-blind placebo-controlled and Phase IV trials. In: *Herpes and Papilloma Viruses* (DePalo, G., Rilke, F., zur Hausen, H., Eds.). Serono Symposia, Vol. 46. Raven Press, New York, pp. 245-259,

Gohji, K., Fidler, I.J., Tsan, R., Radinsky, R., von Eschenbach, A.C., Tsuruo, T., & Nakajima, M. (1994). Human recombinant Interferons-beta and -gamma decrease gelatinase production and invasion by human KG-2 renal-carcinoma cells. Int. J. Cancer 58, 380-384.

Goldstein, D., Sielaff, K.M., Storer, B.E., Brwon, R.R., Datta, S.P., Witt, P.L., Teitelbaum, A.P., Smalley, R.V., & Borden, E.C. (1989). Human biologic response modification by Interferon in the absence of measurable serum concentrations: A comparative trial of subcutaneous and intravenous Interferon-β serine. J. Natl. Cancer Inst. 81, 1061-1068.

Goodburn, S., & Maniatis, T. (1988). Overlapping positive and negative regulatory domains of the human β-Interferon gene. Proc. Natl. Acad. Sci. USA 85, 1447-1451.

Goodburn, S., Zinn, K., & Maniatis, T. (1985). Human β-Interferon gene expression is regulated by an inducible enhancer element. Cell 41, 509-520.

Goodburn, S., Burstein, H., & Maniatis, T. (1986). The human β-Interferon gene enhancer is under negative control. Cell 45, 601-610.

Gothelf, Y., Raber, J., Chen, L., Schattner, A., Chebath, J., & Revel, M. (1991). Terminal differentiation of myeloleukemic M1 cells induced by IL-6: Role of endogenous Interferon. Lymphokine Cytokine Res. 10, 369-375.

Gould, M.N., Kakria, R.C., Olson, S., & Borden, E.C. (1984). Radiosensitization of human bronchogenic carcinoma cells by Interferon-beta. J. Interferon Res. 4, 123-128.

Greenlund, A.C., Farrar, M.A., Viviano, B.L., & Schreiber, R.D. (1994). Ligand-induced IFN-γ receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). EMBO J. 13, 1591-1600.

Gresser, I., Maury, C., Carnaud, S., DeMaeyer, E., Maunoury, M.T., & Bellardelli, F. (1990). Antitumour effects of Interferon in mice injected with IFN sensitive and IFN resistant Friend erythroleukemia cells. VIII. Role of the immune system in inhibition of visceral metastases. Intl. J. Cancer 46, 468-474.

Grion, A.M., Gaion, R.M., Cordella, L., Bano, F., Cannada, R.A., Innamorati, G., & Berti, T. (1994). Interferon-alpha. Results of a pharmaco-epidemiologic study. Clin. Ter. 144, 201-211.

Gross, G., Mayr, U., Bruns, W., Grosveld, F., Dahl, H.H.M., & Collins, J. (1981). The structure of a thirty-six kilobase region of the human chromosome including the fibroblast Interferon gene IFN-beta. Nucleic Acids Res. 9, 2495-2507.

Halme, M., Maasilta, P., Mattson, K., & Cantell, K. (1994). Pharmacokinetics and toxicity of inhaled IFN-β in patients with lung cancer. Respiration 61, 105-107.

Harada, H., Fujita, T., Miyamoto, M., Kimura, Y., Maruyama, M., Furia, A., Miyata, T., & Taniguchi, T. (1989). Structurally similar but functionally distinct factors, IRF-1 and IRF-2 bind to the same regulatory elements of IFN and IFN-inducible genes. Cell 58, 729-739.

Harada, H., Willison, K., Sakakibara, J., Miyamoto, M., Fujita, T., & Taniguchi, T. (1990). Absence of type I IFN system in EC cells: Transcriptional activator (IRF-1) and repressor (IRF-2) genes are developmentally regulated. Cell 63, 303-312.

Harada, H., Kitagawa, M., Tanaka, N., Yamamoto, H., Harada, K., Ishihara, M., & Taniguchi, T. (1993). Anti-oncogenic and oncogenic potentials of Interferon Regulatory Factors-1 and -2. Science 259, 971-974.

Harada, H., Takahashi, E., Itoh, S., Harada, K., Hori, T.A., & Taniguchi, T. (1994). Structure and regulation of the human Interferon Regulatory Factor 1 (IRF-1) and IRF-2 genes: Implications for a gene network in the Interferon system. Mol. Cell. Biol. 14, 1500-1509.

Harroch, S., Gothelf, Y., Watanabe, N., Revel, M., & Chebath, J. (1993). Interleukin-6 activates and regulates transcription factors of the Interferon Regulatory Factor (IRF-1) family in M1 cells. J. Biol. Chem. 268, 9092-9097.

Harroch, S., Revel, M., & Chebath, J. (1994a). Induction by Interleukin-6 of Interferon Regulatory Factor 1 (IRF-1) gene expression through the palindromic Interferon response element pIRE and cell type-dependent control of IRF-1 binding to DNA. EMBO J. 13, 1942-1949.

Harroch, S., Revel, M., & Chebath, J. (1994b). Interleukin-6 signaling via four transcription factors binding palindromic enhancers of different genes. J. Biol. Chem. 269, 26191-26195.

Havell, E.A., Berman, B., Ogburn, C.A., Berg, K., Paucker, K., & Vilcek, J. (1975). Two antigenically distinct species of human Interferon. Proc. Natl. Acad. Sci. USA 72, 2185-2187.

Hawkins, M.J., Krown, SE., Borden, E.C., Krim, M., Real, F.X., Edwards, B.S., Anderson, S.A., Cunningham-Rundles, S., & Oettgen, H.F. (1984). American Cancer Society Phase I trial of naturally produced beta-Interferon. Cancer Res. 44, 5934-5938.

Hayden, F.G., Gwaltney, J.M., & Johns, M.E. (1985). Prophylactic efficacy and tolerance of low-dose intranasal Interferon-α2 in natural respiratory virus infections. Antiviral Res. 5, 111-116.

Hayden, F.G., Innes, D.J., Mills, S.E., & Levine, P.A. (1987). Long term tolerance of intranasal recombinant IFN-β_{SER} in man. In: *The Biology of the Interferon System* (Cantell, K., & Schellekens, H., Eds.). Martinus Nijhoff Publ., Dordrecht, Netherlands, pp 449-456.

Hayes, T.G. (1981). Differences between human α (Leukocyte) and β (Fibroblast) Interferons. Arch. Virol. 67, 267-281.

Hayes, T.G., Yip, Y.K., & Vilcek, J. (1979). *Le* Interferon production by human fibroblasts. Virology 98, 351-363.

Hertzog, P.J., Hwang, S.Y., Holland, K.A., Tymms, M.J., Iannello, R., & Kola, I. (1994). A gene on chromosome 21 located in the region 21q22.2 to 21q22.3 encodes a factor necessary for signal transduction and antiviral resonse to type I Interferons. J. Biol. Chem. 269, 14088-14093.

Higgins, P,G,, Al-Nakib, W., Willman, J., & Tyrell, D.A.J. (1986). Interferon-bser as prophylaxis against experimental rhinovirus infection in voluteers. J. Interferon Res. 6, 153-159.

Hiscott, J., Alper, D., Cohen, L., Leblanc, J.F., Sportza, L., Wong, A., & Xanthoudakis, S. (1989). Induction of human Interferon gene expression is associated with a nuclear factor that interacts with the NF-κB site of the human immunodeficiency virus enhancer. J. Virol. 63, 2557-2566.

Holsti, L.R., Mattson, K., Niiranen, A., Standertsjiold-Noordenstam, C.G., Stenman, S., Sovijarvi, A., & Cantell, K. (1987). Enhancement of radiation effect by alpha interferon in the treatment of small cell carcinoma of the lung. Int. J. Radiation Oncology Biol Phys 13, 1161-1166.

Hoofnagle, J.H., Di Bisceglie, A.M., & Shindo, M.(1993). Antiviral therapy of hepatitis C - present and future. J. Hepatol. 17, S130-S136.

Horisberger, M.A. (1992.) Mx protein: function and mechanism of action. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., Fleischmann, R., Hughes, T.K., Klimpel, G.R., Nielsel, D.W., Stanton, J., & Tyring, S.K. Eds.). The University of Texas Medical Branch at Galveston, Galveston, TX, pp. 215-224.

Hosoi, K., Utsumi, J., Kitagawa, T., Shimizu, H., & Kobayashi, S. (1988). Structural characterization of fibroblast human IFN-β. J. Interferon Res. 8, 375-384.

Huynh, H.K., & Dorovini-Zis, K. (1993). Effects of Interferon-γ on primary cultures of human brain microvessel endothelial cells. Am. J. Pathol. 142, 1265-1278.

Iino, S. (1994). Treatment of chronic hepatitis C with commercial available interferons. Nippon Rinsho 52, 1784-1788.

Improta, T., Schindler, C., Horvath, C.M., Kerr, I.M., Stark, G.R., & Darnell, J.E. (1994). Transcription factor ISGF3 formation requires phosphorylated Stat91 protein, but Stat113 protein is phosphorylated independently of Stat91 protein. Proc. Natl. Acad. Sci. USA 91, 4776-4780.

Jacobs, L.D., O'Malley, J., Freeman, A., & Ekes , R. (1981). Intrathecal Interferon reduces exacerbations of Multiple Sclerosis. Science 214, 1026-1028.

Jacobs, L.D., Salazar, A.M., Herndon, R., Reese, P.A., Freeman, A., Josefowicz, R., Cuetter, A., Husain, F., Smith, W.A., Eres, R., et al. (1986). Multicentre Double-Blind study of intrathecally administered natural human fibroblast Interferon on exacerbations in Multiple Sclerosis. Lancet, 1411-1413.

Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.V., Simon, J.H., et al. (1996). Intramuscular Interferon Beta-1a for disease progression in Relapsing Multiple Sclerosis. Ann. Neurol. 39, 285-294.

James, C.D., He, J., Collins, VP., Allalunis-Turner, M.J., & Day, R.S. (1993). Localization of chromosome 9p homozygous deletions in glioma cell lines with markers constituting a continuous linkage group. Cancer Res. 53, 3674-3676.

Johns, T.G., Mackay, I.R., Callister, K.A., Hertzog, P.J., Devenish, R.J., & Linnane, A.W. (1992). Antiproliferative potencies of Interferons on melanoma cell lines

and xenografts: Higher efficacy of Interferon β. J. Natl. Cancer Inst. 84, 1185-1190.

Joseph, J. Knobler, R.L., D'Imperio, C., & Lublin, F.D. (1988). Down regulation of IFN-γ induced MHC class II expression on human glioma cells by recombinant IFN-β: Effects of dosage treatment schedule. J. Neuroimmunol. 20, 39-44.

Kagawa, Y. Takasaki, S., Utsumi, J., Hosoi, K., Shimizu, H., Kochibe, N., & Kobata, A. (1988). Comparative study of the asparagine-linked sugar chains of natural human Interferon-β1 and recombinant human Interferon-β1 produced by three different mammalian cells. J. Biol. Chem. 263, 17508-17515.

Kagawa, T., Morizane, T., Saito, H., Tsunematsu, S., Tada, S., Kumagai, N., Tsuchimoto, K., Sugiura, H., Mukai, M., & Tsuchiga, M. (1993). A pilot study of long-term weekly interferon-beta administration for chronic hepatitis B. Am. J. Gastroenterol. 88, 212-216.

Kakumu, S., Yoshioka, K., Wakita, T., Ishikawa, T., Takayanagi, M., & Higashi, Y. (1993). A pilot study of ribavirin and interferon-beta for the treatment of chronic hepatitis C. Gastroenterol. 105, 507-512.

Kanda, Y., Shigeno, K., Kinoshita, N., Nakao, K., Yano, M., & Matsuo, H. (1994) Sudden hearing loss associated with Interferon. Lancet 343, 1134-1135.

Kanno, Y., Kozak, C.A., Schindler, C., Driggers, P.H., Ennist, D.L., Gleason, S.L., Darnell, J.E., & Ozato, K. (1993). The genomic structure of the murine ICSBP gene reveals the presence of the gamma Interferon-responsive element, to which an ISGF3 alpha subunit (or similar) molecule binds. Mol. Cell. Biol. 13, 3951-3963.

Kaplowitz, L.G., Baker. D., Gelb. L., Blythe, J., Hale, R., Frost, P., Crumpacker, C., Rabinovich, S., Peacock, J.E., Herndon, J., Davis, G., & the Acyclovir Study Group. (1991). Prolonged continuous acyclovir treatment of normal adults with frequently recurring genital herpes simplex virus infection. JAMA 265, 745-751.

Keilholz, U., Scheibenbogen, C., Stoelben, E., Saeger, H.D., & Hunstein, W. (1994). Immunotherapy of metastatic melanoma with interferon-alpha and interleukin-2: Pattern of progression in responders and patients with stable disease with or without resection of residual lesions. Eur. J. Cancer 30A, 955-958.

Keller, A.D., & Maniatis, T. (1991). Identification and characterization of a novel repressor of β-Interferon gene expression. Genes Dev. 5, 868-879.

Keller, A.D., & Maniatis, T. (1992). Only two of the five zinc fingers of the eukaryotic transcriptional repressor PRDI-BF1 are required for sequence-specific DNA binding. Mol. Cell. Biol. 12, 1940-1949.

Kent, H.L., & Wisniewski, P.M. (1990). Interferon for vulvar vestibulitis. J. Reprod. Med. 12, 1138-1143.

Kimura, S., Utsumi, J., Yamazaki, S., & Shimizu, H. (1988). Disulfide bond interchange in *Escherichia coli*-derived recombinant human interferon-beta 1 under denaturing conditions. J. Biochem. (Tokyo) 104, 44-47.

Kimura, M., Majde, J.A., Toth, L.A., Opp, M.R., & Krueger, J.M. (1994). Somnogenic effects of rabbit and recombinant human interferons in rabbits. Am. J. Physiol. 267, R53-61.

Kirchoff, S,, Schaper, F., & Hauser, H. (1993). Interferon Regulatory Factor 1 (IRF-1) mediates cell growth inhibition by transactivation of downstream target genes. Nucleic Acids Res. 21, 2881-2889.

Kirkwood, J.M. (1994). Systemic therapy of melanoma. Curr. Opin. Oncol. 6, 204-211.

Kishimoto, T., Taga, T., & Akira. S. (1994). Cytokine signal transduction. Cell 76, 253-262.

Kiyosawa, K., Sodeyama, T., Nakano, Y., Yoda, H., Tanaka, E., Hayata, T., Tsuchya, K., Yousuf, M., & Furuta, S. (1989). Treatment of chronic non-A non-B hepatitis with human interferon β: A preliminary study. Antiviral Res. 12, 151-162.

Knabbe, C., Lippmanm M,E,, Wakefield, L.M., Flanders, K.C., Kasid, A., Derynck, R., & Dickson, R.B. (1987). Evidence that transforming growth factor-β is a hormonallly regulated negative growth factor in human breast cancer cells. Cell 48, 417-428.

Knight, E. (1981). Purification of human fibroblast Interferon prepared in the absence of serum. Meth. Enzymol. 78, 417-421.

Knight, E., & Fahey, D. (1982). Human Interferon β: Effect of deglycosylation. J. Interferon Res. 2, 421-429.

Knight, E., Hunkapillar, M.W., Korant, B.D., Hardy, R.W.F., & Hood, L.E. (1980.) Human fibroblast interferon: Amino acid analysis and amino terminal amino acid sequence. Science 207, 525-526.

Knobler, R.L., Greenstein, J.I., Johnson, K.P., Lublin, F.D., Panitch, H.S., Conway, K., Grant-Gorsen, S.V., Muldoon, J., Marcus, S.J., Wallenberg, J.C., Williams, G.J., & Yoshizawa, C.N. (1993). Systemic recombinant human Interferon-beta treatment of relapsing-remitting multiple sclerosis: Pilot study analysis and six-year follow-up. J. Interferon Res. 13, 333-340.

Kobayashi, Y., Watanabe, S., Konishi, M., Yokoi, M., Kakehashi, R., Kaito, M., Kondo, M., Hayashi, Y., Jomori, T., & Suzuki, S. (1993). Quantitation and typing of serum hepatitis C virus RNA in patients with chronic hepatitis C treated with interferon-beta. Hepatology 18, 1319-1325.

Korn, A.P., Rose, D.R., & Fish, E.N. (1994). Three-dimensional model of a human Interferon-α consensus sequence. J. Interferon Res. 14, 1-9.

Kraus, E., Scneider-Schaulies, S., Miyasaka, M., Tamanati, T., & Sedwick, J. (1992). Augmentation of major histocompatibility complex class I and ICAM-1 expression on glial cells following measles virus infection: Evidence for the role of type I Interferon. Eur. J. Immunol. 22, 175-182.

Krigel, R.L., Padavic-Shaller, K.A., Rudolph, A.R., Litwin, S., Konrad, M., Bradley, E.C., & Cosmis, R.L. (1988). A phase I study of recombinant interleukin-2 plus recombinant IFN-β. Cancer Res. 48, 3875-3881.

Kumar, A., Haque, J., Lacoste, J., Hiscott, J., & Williams, B.R.G. (1994). Double-stranded RNA-dependent protein kinase activates transcription factor NF-κB by phosphorylating I kappa B. Proc. Natl. Acad. Sci. USA 91, 6288-6292.

Kuo, Y.C., & Lin, C.Y. (1990). Recurrent herpes simplex virus type 1 infections precipitated by the impaired production of Interleukin-2, alpha-Interferon and cell-mediated cytotoxicity. J. Med. Virol. 31, 183-189.

Leblanc, J.F., Cohen, L., Rodrigues, M., & Hiscott, J. (1990). Synergism between distinct enhanson domains in viral induction of the human beta Interferon gene Mol. Cell. Biol. 10, 3987-3993.

Lenardo, M.J., Fan, C-M., Maniatis, T., & Baltimore, D. (1989). The involvement of NF-κB in β-Interferon gene regulation reveals its role as a widely inducible mediator of signal transduction. Cell 57, 287-294.

Lengyel, P. (1993). Tumor-suppressor genes: News about the Interferon connection. Proc. Natl. Acad. Sci. USA 90, 5893-5895.

Leong, S.S., & Horoszewicz, J.S. (1981). Production and preparation of human fibroblast Interferon for clinical trials. Meth. Enzymol. 78, 87-101.

Levi, H.B., & Salazar, A.M. (1992). Interferon inducers. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., et al., Eds.). The University of Texas Medical Branch at Galveston, Galveston, TX, pp. 65-76.

Levin, M.J., Judson, F.N., Eron, L., Bryson, Y.J., Corey, L., Murray, M., & Scheer, R.R. (1989). Comparison of intramuscular recombinant alpha Interferon (rIFN-α2a) with topical acyclovir for the treatment of first-episode herpes genitalis and prevention of recurrences. Antimicrob. Agents Chemother. 33, 649-652.

Liberati, A.M., Fizzotti, M., Proietti, M.G., DiMarzio, R., Schippa, M., Biscottini, B., Senatore, M., Berruto, P., Canali, S., Peretti, G., & Zanolo, G. (1988). Biochemical host response to Interferon-β. J. Interferon Res. 8, 765-777.

Liberati, A.M., Biscottini, B., Fizzotti, M., Schippa, M., DeAngelis, V., Senatore, M., Vittori, O., Teggia, L., Natali, R., Palmisano, L., & Canali, S. (1989). A phase I study of human natural IFN-β in cancer patients. J. Interferon Res. 9, 339-348.

Liberati, A.M., Biagini, S., Perticoni, G., Ricci, S., D'Alessandro, P., Senatore, M., & Cinieri, S. (1990a). Electrophysiological and neuropsychological functions in patients treated with IFN-β. J. Interferon Res. 10, 613-619.

Liberati, A.M., Fizzotti, M., DiClemente, F., Senatore, M., Berruto, P., Falini, B., Martelli, M.F., & Grignani, F. (1990b). Response to intermediate and standard doses of IFN-β in hairy cell leukemia. Leukemia Res. 14, 779-784.

Liberati, A.M., Horisberger, M., Schippa, M., DiClemente, F., Fizzotti, M., Filippo, S., Proietti, M.G., Arzano, P., Berruto, P., Palmisano, L., & Cinieri, S. (1991). Biochemical and immunological responses of hairy cell leukemia patients to Interferon-β. Cancer Immunol. Immunother. 34, 115-122.

Liberati, A.M., Horisberger, M.A., Palmisano, L., Astolfi, S., Nastari, A., Mechati, S., Villa, A., Mancini, S., Arzano, S., & Grignani, F. (1992). Double blind randomized Phase I study on the clinical tolerance and biological effects of natural and recombinant IFN-β . J. Interferon Res. 12, 329-336.

Liberati, A.M., De Angelis, V., Fizzotti, M., Schippa, M. Cecchini, M., Adiuto, D., Diclemente, F., Palmisano, L., Micozzi, E., Zuccaccia, M., et al., (1994a). Natural killer stimulatory effect of combined low-dose Interleukin-2 and Interferon-beta in Hairy cell leukemia patients. Cancer Immunol. Immunother. 38, 323-331.

Liberati, A.M., Garofani, P., De Angelis, V., Diclemente, F., Horizberger, M., Cecchini, M., Betti, A.R., Palmisano, L., Astolfi, S., Nastari, A., Villa, A., & Arzano, S. (1994b). Double-blind Randomized Phase I study on the Clinical tolerance and pharmacodynamics of natural and recombinant IFN-β given intravenously. J. Interferon Res. 14, 61-69.

Lin, H.Y., Davis, P.J., & Thacore, H.R. (1991). Production of human Interferon-β by Sendai virus and poly rI:rC: Inhibition by neomycin. J. Interferon Res. 11, 365-369.

Lingwood, C.A., & Shiu, S.K. (1992). Glycolipid modification of alpha2 Interferon binding. Biochem. J. 283, 25-26.

Lu, H.T., Riley, J.L., Babcock, G.T., Huston, M., Stark, G.R., Boss, J.M., & Ransohoff, R.M. (1995). Interferon (IFN) beta acts downstream of IFN-gamma-induced class

II transactivator messenger RNA accumulation to block major histocompatability complex class II gene expression and requires the 48-kD DNA-binding protein, ISGF3-gamma. J. Exp. Med. 182, 1517-1525.

Lucero, M.A., Fridman, W.H., Provost, M.A., Billardon, C., Pouillart, P., Dumont, J., & Falcoff, E. (1981). Effect of various Interferons on the spontaneous cytotoxicity exerted by lymphocytes from normal and tumor-bearing patients. Cancer Res. 41, 294-300.

Lucero, M.A., Magdalenat, H., Fridman, W.H., Pouillart, P., Billardon, C., Billiau, A., Cantell, K., & Falcoff, E. (1982). Comparison of effects of leukocytes and fibroblast Interferon on immunological parameters in cancer patients. Eur. J. Cancer Clin. Oncol. 18, 243-251.

Lutfalla, G., Holland, S.J., Cinato, E., Monneron, D., Reboul, J., Rogers, N.C., Smith, J.M., Stark, G.R., Gardiner, K., Mogensen, K.E., Kerr, I.M., & Uze, G. (1995). Mutant U5A cells are complemented by an interferon-αβ receptor subunit generated by an alternative processing of a new member of a cytokine receptor gene cluser. EMBO J., 14, 5100-5108.

MacDonald, N.J., Kuhl, D., Maguire, D., Naf, D., Gallant, P., Goswami, A., Hug, H., Bueler, H., Chaturvedi, M., de la Fuente, J., Ruffner, H., Meyer, F., & Weissmann, C. (1990). Different pathways mediate virus inducibility of the human IFN-α 1 and IFN-β genes. Cell 60, 767-779.

Macheledt, J.E., Buzdar, A.U., Hortobagyi, G.N., Frye, D.K., Gutterman, J.U., & Holmes, F.A. (1991). Phase II evaluation of interferon added to tamoxifen in the treatment of metastatic breast cancer. Breast Cancer Res. Treat 18, 165-170.

Maffei Faccioli, A., Gerunda, G.E., Neri, D., Zangrandi, F., Merenda, R., Bruttocao, A., Medwri, F., Barbazza, F., Bisello, M., Dimarzio, E., et al. (1994). The palliative treatment of hepatocarcinoma: Chemoembolization versus the combination of Tamoxifen plus beta-Interferon. Chir. Ital. 46, 12-16.

Marcus, P.I. (1983). Interferon induction by viruses: One molecule of ds RNA as the threshold for Interferon induction. In: *Interferon 5* (Gresser, I., Ed.). Academic Press, London, pp. 115-180.

Marcus, P.I. (1987). Personal travels and travails with the Interferon system. J. Interferon Res. 7, 471-479.

Mark, D.F., Lu, S.D., Creasey, A.A., Yamamoto, R., & Lin, L.S. (1984). Site-specific mutagenesis of the human fibroblast interferon gene. Proc. Natl. Acad. Sci. USA 81, 5662-5666.

Markowska, J., Nowak, M., Niecewicz, R., Breborowicz, J., Wiese, E., Zengteler, G. (1994). Results of topical treatment of HPV infection in the uterine cervix using interferon beta, 13-cis-retinoic acid and TFX. Eur. J. Gynaecol. Oncol. 15, 56-69.

Matsushita, E., Unoura, M., & Kobayashi, K. (1994). Psychiatric complications of IFN therapy. Nippon-Rinsho 52, 1910-1913.

Matsuyama, T., Kimura, T., Kitagawa, M., Pfeffer, K. et al., (1993). Targeted disruption of IRF-1 or IRF-2 results in abnormal Type I IFN gene induction and aberrant lymphocyte development. Cell 75, 83-97.

Mattson, K., Niiranen, A., Laaksonen, R., & Cantell, K. (1984). Psychometric monitoring of interferon neurotoxicity. Lancet 1, 275-276.

McDonald, S., Chang, A.Y., Rubin, P., Wallenberg, J., Kim, I.S., Sobel, S., Smith, J., Keng, P., & Muhs, A. (1993). Combined Betaseron R (Recombinant human Interferon beta) and radiation for inoperable Non-small Cell Lung Cancer. Int. J. Radiation Oncology Biol. Phys. 27, 613-619.

Meager, A., Graves, H.E., Shuttleworth, J., & Zucker, N. (1979). Interferon production: variation in yields from human cell lines. Infect. Immun. 25, 658-663.

Merlin, G., Falcoff, E., & Aguet, M. (1985). ^{125}I-labeled human Interferon alpha, beta and gamma: Comparative receptor binding data. J. Gen. Virol. 66, 1149-1152.

Meurs, E.F., Watanabe, Y., Kadereit, S., Barber, G.N., Katze, M.G., Chong, K., Williams, B.R., & Hovanessian, A.G. (1992). Constitutive expression of human double-stranded RNA-activated p68 kinase in murine cells mediates phosphorylation of eukaryotic initiation factor 2 and partial resistance to encephalomyocarditis growth. J. Virol. 66, 5804-5814.

Michalevicz, R., & Revel, M. (1987). Interferons regulate the in vitro differentiation of mulltilineage lympho-myeloid stem cells in hairy cell leukemia. Proc. Natl. Acad. Sci. USA 84, 2307-23011.

Michalevicz, R., Aderka, D., Frisch, B., & Revel, M. (1988). Interferon-beta induced remission in a hairy cell leukemia patient resistant to Interferon-alpha. Leukemia Res. 12, 845-851.

Mihara, Y., Kuratsu, J., Takaki, S., Hori, K., Nagai, E., Satoh, Y., Minowa, N., & Ushio, Y. (1991). Distribution of mouse Interferon-beta in normal and brain tumour-bearing mice. Acta Neurochir. 109, 46-51.

Milella, M., Antonelli, G., Santantonio, T., Currenti, M., Monno, L., Mariano, N., Angarano, G., Dianzani, F., & Pastore, G. (1993). Neutralizing antibodies to recombinant alpha-interferon and response to therapy in chronic hepatitis C virus infection. Liver 13, 146-150.

Miles, S.A., Wang, H., Cortes, E., Carden, J., Marcus, S., & Mitsuyasu, R.T. (1990). Beta-Interferon therapy in patients with poor diagnostic Kaposi's sarcoma related to the acquired immunodeficiency syndrome (AIDS). Ann. Intern Med. 112. 582-589.

Miller, A., Lanir, N., Shapiro, S., Revel, M., Honigman, S., Kinarty, A., & Lahat, N. (1996). Immunoregulatory effects of Interferon-β and interacting cytokines on human microvessel endothelial cells. J. Neuroimmunol. 64, 151-156.

Mitsui, Y., Senda, T., Shimazu, T., Matsuda, S., & Utsumi, J. (1993). Structural, functional and evolutionary implications of the three-dimensional crystal structure of murine Interferon-beta. Pharmacol. Ther. 58, 93-132.

Miyamoto, M., Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T., & Taniguchi, T. (1988). Regulated expression of a gene encoding a nuclear factor, IRF-1 that specifically binds to IFN-β gene regulatory elements. Cell 54, 903-913.

Molto, L., Carballido, J., Manzano, L., Olivier, C., Lapena, P., & Alvarez-Mon, M. (1994). Interferon-beta enhances the natural killer activity of patients with bladder carcinoma. Cancer Immunol. Immunother. 38, 406-410.

Mory, Y., Chernajovsky Y., Feinstein, S.I., Chen, L., Nir, U., Weissenbach, J., Malpiece, Y., Tiollais, P., Marks, D., Ladner, M., Colby, C., & Revel, M. (1981). Synthesis of human Interferon β in *Escherichia Coli* by a Lambda phage recombinant containing a human genomic fragment. Eur. J. Biochem. 120,, 197-202.

Mosmann, T.R., & Moore, K.W. (1991). The role of IL-10 in cross-regulation of TH1 and TH2 responses. Immunol. Today 12, (3), A49-A53.

Movshovitz, M., Schewach-Millet, M., Kriss-Leventon, S., Shoham, J., Doerner, T.T., & Revel, M. (1985). Topical treatment of recurrent facial and genital herpes with human Interferon beta cream. In: *Herpes Viruses and Virus Chemotherapy* (Kono, R., & Nakajima, A., Eds.). Elsevier Scientific Publishers, Amsterdam, pp. 285-292,

Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A.G., Barbieri, G., Witthuhn, B.A., Schindler, C., Pellegrini, S., Wilks, A.F., Ihle, J.N., Stark, G.A., & Kerr, I.M. (1993). The protein tyrosine kinase JAK1 complements defects in Interferon alpha, beta and gamma signal transduction. Nature 366, 129-135.

Naf, D., Hardin, S.E., & Weissmann, C. (1991). Multimerization of AAGTGA and GAAAGT generates sequences that mediate virus inducibility by mimicking an Interferon promoter element. Proc. Natl. Acad. Sci. USA 88, 1369-1373.

Nagai, M. (1988). Clinical use of Interferons in the treatment of malignant brain tumors. In: *Clinical Aspects of Interferons* (Revel, M., Ed.). Kluwer Academic Publishers, Boston, pp. 183-193.

Nagai, M. (1991). Advances of BRM therapy of malignant brain tumors. Gan To Kagaku Ryoho 18, 188-194.

Nagai, J., & Arai, T. (1984). Clinical effect of Interferon in malignant brain tumors. Neurosurg. Rev. 7, 55-64.

Nagashima, H., Arima, T., & Suzuki, H. (1987). Treatment of chronic non-A non-B hepatitis with human interferon-beta. J. Med. Virol. 21, 128a.

Nair, S., Mayotte, J., Lokshin, A., & Levitt, M. (1994). Induction of squamous differentiation by interferon-beta in a human non-small cell lung cancer cell line. J. Natl. Cancer Inst. 86, 378-383.

Nakayama, J., Toyofuku, K., Urabe, A., Taniguchi, S., & Hori, Y. (1993). A combined therapeutic modality with hyperthermia and locally administered rIFN-beta inhibited the growth of B16 melanoma in association with the modulation of cellular infiltrates. J. Dermatol. Sci. 6, 240-246.

Namba, M., Yamamoto, S., Tanaka, H., Kanamori, T., Nobuhara, M., & Kimoto, T. (1984). *In vitro* and *in vivo* studies on potentiation of cytotoxic effects of anti-cancer drugs or cobalt-60 gamma ray by interferon on human neoplastic cells. Cancer 54, 2262-2267.

Nanni, P., Landuzzi, L., Nicoletti, G., DeGiovanni, M., Lalli, E., Facchini, A., & Lollini, P.L. (1992). Control of H-2 expression in transformed nonhaematopoietic cells by autocrine Interferon. Br. J. Cancer 66, 479-482.

Neefe, J,R., & John, W. (1991). Mechanisms of interaction of Interferon and 5-fluorouracil in solid tumors. Semin. Oncol. 18, 77-82.

Nir, U., Cohen, B., & Revel, M. (1984). A human IFN-β1 gene deleted of promoter sequences upstream from TATA box is controlled post-transcriptionally by ds RNA. Nucleic Acids Res. 12, 6979-6993.

Nishiguchi, S., Kuroki, T., Otani, S., Takeda, T., Hirota, S., Shimizu, Y., Nakajima, S., Saito, S., Shiomi, S., & Kobayashi, K. (1989). Relationship of the effect of Interferon on chronic hepatitis B and the induction of 2',5'-oligoadenylate synthetase. Hepatology 10, 29-33.

Nokta, M., Loh, J.P., Douidar, S.M., Ahmed, A.E., & Pollard, R.B. (1991). Metabolic interaction of recombinant Interferon-β and Zidovudine in AIDS patients. J. Interferon Res. 11, 159-164.

Noronha, A., Toscas, A., & Jensen, M.A. (1992). Contrasting effects of alpha, beta and gamma Interferons on nonspecific suppressor cell functions in multiple sclerosis. Ann. Neurol. 31, 103-106.

Novick, D., Cohen, B., & Rubinstein, M. (1994). The human Interferon α/β Receptor: Characterization and molecular cloning. Cell 77, 391-400.

Ohnishi, K., Nomura, F., & Nakano, M. (1991). Interferon therapy for acute posttransfusion non-A non-B hepatitis: Response with respect to anti-hepatitis C virus. Am. J. Gastroenterol. 86, 1041-1049.

Oka, S., Hirabayashi, Y., Mouri, H., Sakurada, S., Goto, H., Ohnishi, K., Kimura, S., Mitamuram K., & Shimada, K. (1989). Beta-Interferon and early stage HIV infection. J. Acquir. Immune Defic. Syndr. 2, 125-128.

Olmos, L., Vilatam, J., Rodriguez Pichardo, A., Lloret, A., Ojeda, A., & Calderon, M.D. (1994). Double-blind, randomized clinical trial on the effect of interferon-beta in the treatment of condylomata acuminata. Int. J. STD AIDS 5, 182-185.

Omata, M., Yokosuka, O., Takano, S., Kato, N., Hosoda, K., Imazeki, F., Tada, M., Ito, Y., & Ohto, M. (1991). Resolution of acute hepatitis C after therapy with natural beta-interferon. Lancet 338, 914-915.

Ophir, J., Brenner, S., Bali, R., Kriss-Leventon, S., Smetana, Z., & Revel, M. (1995). Effect of topical Interferon-beta on recurrence rates in genital herpes: A double-blind, placebo-controlled, randomized study. J. Interferon Cytokine Res. 15, 625-631.

Oriel, J.D. (1983). Condylomata acuminata as a sexually transmitted disease. Dermatol. Clin. 1, 93-102.

O'Rourke, E.C., Drummond, R.J., & Creasey, A.A. (1984) Binding of ^{125}I-labeled recombinant β Interferon (IFN-β Ser17) to human cells. Mol. Cell. Biol. 4, 2745-2749.

Pagliaci, M.C., Pelicci, G., Schippa, M., Liberati, A.M., & Nicoletti, I. (1991). Does IFN-β therapy induce thyroid autoimmune phenomena? Horm. Metab. Res. 23, 196-197.

Palmer, H., & Libby, P. (1992). Interferon beta. A potential autocrine regulator of human vascular smooth muscle cell growth. Lab. Invest. 66, 715-721.

Palombella, V.J., & Maniatis, T. (1992). Inducible processing of Interferon Regulatory Factor-2. Mol. Cell. Biol. 12, 3325-3336.

Panitch, H.S. (1994). Influence of infection on exacerbations of multiple sclerosis. Ann. Neurol. 36, S25-28.

Panitch, H.S., & Bever, C.T. (1993). Clinical trials of Interferons in Multiple Sclerosis: What have we learned. J. Neuroimmunol. 46, 155-164.

Panitch, H.S., Hirsch, R.L., Schindler, J., & Johnson, K.P. (1987). Treatment of multiple sclerosis with gamma Interferon: Exacerbations associated with activation of the immune system. Neurology 37, 1097-1102.

Paty, D.W., Li, D.K.B., the UBC MS/MRI Study Group, & the IFNB Multiple Sclerosis Study Group. (1993). Interferon beta-1b is effective in relapsing remitting Multiple Sclerosis. II. MRI analysis results of a multicenter, randomized, double blind, placebo-controlled trial. Neurology 43, 662-667.

Paty, D.W., Li, D.K., Oger, J.J., Kastrukoff, L., Koopmans, R., Tanton, E., & Zhao, G.J. (1994). Magnetic resonance imaging in the evaluation of clinical trials in Multiple Sclerosis. Ann. Neurol. 36, S95-S96.

Paulesu, L., Pessina, G.P., & Bocci, V. (1992). Human beta-Interferon incubated with muscle homogenate is protected by albumin but not by proteinase inhibitors. Proc. Soc. Biol. Med. 200, 414-417.

Pellegrini, S., & Schindler, C. (1993). Early events in signalling by Interferons. Trends Biochem. Sci. 18, 338-342.

Penna, C., Fallani, M.G., Gordigiani, R., Sonni, L., Taddei, G.L., & Marchionni, M. (1994). Intralesional beta-interferon treatment of cervical intraepithelial neoplasia associated with human papillomavirus infection. Tumori 80, 146-150.

Perrillo, R.P., & Mason, A.L. (1994). Therapy for hepatitis B infection. Gastroenterol. Clin. North Am. 23, 581-601.

Pestka, S., Langer, J., Zoon, K., & Samuel, C.E. (1987). Interferons and their actions. Ann. Rev. Biochem. 56, 727-777.

Pine, R. (1992). Constitutive expression of an ISGF2/IRF-1 transgene leads to Interferon-independent activation of Interferon-inducible genes and resistance to virus infection. J. Virol. 66, 4470-4478.

Pine, R., Levy, D.E., Reich, N., & Darnell, J.E. (1990). Purification and cloning of Interferon-stimulated gene factor 2 (ISGF2): ISGF2 (IRF-1) can bind to the promoters of both beta Interferon and Interferon stimulated genes but not a primary transcriptional activator of either. Mol. Cell. Biol. 10, 2448-2457.

Pitha, P.M. (1991). Multiple effects of Interferon on HIV-1 replication. J. Interferon Res. 11, 313-318.

Platanias, L.C., & Colamonici, O.R. (1992). Interferon-α induces rapid tyrosine phosphorylation of the α-subunit of its receptor. J. Biol. Chem. 267, 24053-24057.

Platanias, L.C., Uddin, S., & Colamonici, O.R. (1994). Tyrosine phosphorylation of the α and β subunits of the type I Interferon Receptor. Interferon-b selectively induces tyrosine phosphorylation of an a subunit-associated protein. J. Biol. Chem. 269, 17761-17764.

Platanias, L.C., Uddin, S., Domanski, P., & Colamonici, O.R. (1996). Differences in Interferon α and β signaling: Interferon-β selectively induces the interaction of the α and β_L subunits of the type I Interferon receptor. J. Biol. Chem., 271.

Poitrine, A., Chousterman, S., Chousterman, M., Naveau, S., Thang, M.N., & Chaput, J.C. (1985). Lack of *in vivo* activation of the interferon system in HBs-Ag-poitive chronic active hepatitis. Hepatology 5, 171-174.

Porrini, A.M. & Reder, A.T. (1994). IFN-γ, IFN-β and PGE1 affect monokine secretion: Relevance to monocyte activation in multiple sclerosis. Cell Immunol. 157, 428-438.

Porrini, A.M., Gambi, D., & Reder, A.T. (1995). Interferon effects on Interleukin-10 secretion. Mononuclear cell response to Interleukin-10 is normal in multiple sclerosis patients. J. Neuroimmunol. 61, 27-34.

Porzsolt, F., Otto, A.M., Trauschel, B., Buck, C., Wawer, A.W., & Schoenenberger. H. (1989). Rationale for combining tamoxifen and interferon in the treatment of advanced breast cancer. J. Cancer Res. Clin. Oncol. 115, 465-469.

Pouillart, P., Palangie, P., Jouve, M., Garcie-Giralt, E., Fridman, W.H., Magdelenat, H., Falcoff, E., & Billiau, A. (1982). Administration of fibroblast interferon to patients with advanced breast cancer: Possible effect

on skin metastasis and on hormone receptors. Eur. J. Cancer Clin. Oncol. 18, 929-935.

Poutanien, E., Hokkanen, L., Niemi, M.L., & Farkkila, M. (1994). Reversible cognitive decline during high dose alpha-interferon treatment. Pharmacol. Biochem. Behav. 47, 901-905.

Pozzilli, C., Bastianello, S., Koudriavtseva, T., Gasperini, C., Bozzao, A., Millefiorini, E., Galgani, S., Buttinelli, C., Perciaccante, G., Piazza, G., Bozzao,, L., & Fieschi, C. (1996). Magnet resonance imaging changes with recombinant IFN-β1-a: A short term study in relapsing remitting multiple schlerosis. J. Neurol. BNeurosurg. Psych. 61, 251-258.

Quesada, J.R. (1992). Toxicity and side-effects of Interferons. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., Fleischmann, R., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Stanton, J., & Tyring, S.K. Eds.). The University of Texas Medical Branch at Galveston, pp. 427-432.

Raj, N.B.K., & Pitha, P.M. (1980). Synthesis of new proteins associated with the induction of Interferon in human fibroblast cells. Proc. Natl. Acad. Sci. USA 77, 4918-4922.

Raj, N.B.K., & Pitha, P.M. (1983). Two levels of regulation of β-Interferon gene expression in human cells. Proc. Natl. Acad. Sci. USA 80, 3923-3927.

Rani, S.M.R., Gauzzi, C., Pellegrini, S., & Ransohoff, R.M. (1996). A functional Tyk2 kinase is required for induction of the β-R1 gene. Eur. Cytokine Netw. 7, 531 (abst. 283).

Rapp, F. (1988), Herpes viruses, venereal disease and cancer. American Scientist 66, 670-674.

Raziuddin, A., Sarkar, F.H., Dutkowski, R., Shulman, L.M., Ruddle, F.H., & Gupta, S.L. (1984). Receptors for human α and β Interferon but not for γ Interferon are specified by human chromosome 21. Proc. Natl. Acad. Sci. USA 81, 5504-5508.

Recchia, F., Sica, G., de Filippis, S., Discepoli, S., Rea, S., Torchio, P., & Frati, L. (1995). Interferon-beta, retinoids, and tamoxifen in the treatment of metastatic breast cancer: A phase II study. J. Interferon Cytokine Res. 15, 605-610.

Reichman, R.C., Oakes, D., Bonnez, W., Greisberger, C., Tyring, S., Miller, L., Whitley, R., Carveth, H., Weidner, H., Krueger, G., et al. (1988). Treatment of condyloma acuminatum with three different interferons administered intralesionally. Ann. Int. Med. 108, 675-679.

Reis, L.F., Harada, H., Wolchok, J.D., Taniguchi, T., & Vilcek, J. (1992). Critical role of a common transcription factor, IRF-1, in the regulation of IFN-β and IFN-inducible genes. EMBO J. 11, 185-193.

Renault, P.F., Hoofnagle, J.H., Park, Y., Mullen, K.D., Peters, M., Jones, D.B., Rustgi, V., & Jones, E.A. (1987). Psychiatric complications of long-term interferon alfa therapy. Arch. Intern. Med. 147, 1577-1580.

Repetto, L., Venturino, A., Simoni, C., Rosso, M., Meliolo, G., & Rosso, R. (1993). Interferons in the treatment of advanced breast cancer. J. Biol. Regul. Homeost. Agents 7, 109-114.

Resnitzky, D., Yarden, A., Zipori, D., & Kimchi, A. (1986). Autocrine β-related Interferon controls c-myc suppression and growth arrest during hematopoietic cell differentiation. Cell 46, 31-40.

Revel, M. (1983). Genetic and functional diversity of Interferons in man. In: *Interferon 5* (Gresser, I., Ed.). Academic Press, New York, pp. 205-239.

Revel, M. (1984). The Interferon system in man: Nature of the Interferon molecules and mode of action. In: *Antiviral Drugs and Interferon: The Molecular Basis of Their Activity* (Becker, Y., Ed.). Martinus Nijhoff Publishers, Boston, pp. 357-433.

Revel, M., & Chebath, J. (1986). Interferon-activated genes. Trends Biochem. Sci. 11, 166-170.

Revel, M., Basch, D., & Ruddle, F.H. (1976). Antibodies to a cell surface component coded by human chromosome 21 inhibit action of Interferon. Nature 260, 139-141.

Revel, M., Schattner, A., Wallach, D., Merlin, G., Levavi, H., Hahn, T., & Levin, S. (1981). Monitoring of Interferon therapy, diagnosis of viral diseases and detection of Interferon deficiencies by assay of an Interferon-induced enzyme in human peripheral white blood cells. In: *The Clinical Potential of Interferons* (Kono, R., & Vilcek, I., Eds.). University of Tokyo Press, Tokyo, pp. 353-367.

Revel, M., Zilberstein, A., Ruggieri, R.M., Rubinstein, M., & Chen, L. (1987). Autocrine Interferons and Interferon-β_2. J. Interferon Res. 7, 529-536.

Revel, M., Chebath, J., Mangelus, M., Harroch, S., & Moviglia, G. (1995). Antagonism of Interferon-beta on Interferon-gamma: Inhibition of signal transduction *in vitro* and reduction of serum levels in multiple sclerosis patients. Multiple Sclerosis 1, S5-S11.

Ringold, G.M., Dieckmann, B., Vannice, J.L., Trahey, M., & McCormick, F. (1984). Inhibition of protein synthesis stimulates the transcription of human β-Interferon genes in chinese hamster ovary cells. Proc. Natl. Acad. Sci. USA 81, 3964-3968.

Romano, A., & Sadan, Y. (1988). Ten years of experience with human fibroblast Interferon in treatment of viral ophtalmic infections. Metb. Pediatr. Syst. Ophtalmol. 11, 43-46.

Romano, A., Revel. M., Gurari Rotman, D., Blumenthal, M., & Stein, R. (1980). Use of fibroblast-derived Beta-Interferon in the treatment of epidemic adenovirus keratoconjunctivitis. J. Interferon Res. 1, 95-100.

Rosa, F.M., Cochet, M.M., & Fellous, M. (1986). Interferon and Major Histocompatibility complex genes: A model to analyse eukaryotic gene regulation? In: *Interferon 7* (Gresser, I., Ed.). Academic Press, London, pp. 47-87.

Rosenblum, M.G., Yung, W.K.A., Kelleher, P.J., Ruzicka, F., Steck, P.A., & Borden, E.C. (1990). Growth inhibitory effects of Interferon-β but not Interferon-α on human glioma cells: Correlation of receptor binding, 2'-5'-oligoadenylate synthetase and protein kinase activity. J. Interferon Res. 10, 141-151.

Rossiello, F., DeCicco-Nardone, F., & DellAcqua, S. (1994). Interferon-beta increases the sensitivity of endometrial cancer cells to cell-mediated cytotoxicity. Gynecol. Oncol. 54, 130-136.

Rosso, R., Nobile, M.T., Sertolim M.R., Giannitelli, A., Santi, P.L., Volpe, R., & Nicolo, G. (1985). Antitumoral activity of human fibroblast Interferon administered intranodularly. Oncology 42, 86-88.

Rosso, R., Rimoldi, R, Salvati, F., De Palma, M., Cinquegrana, A., Nicolo, G., Ardizzoni, A., Fusco, U., Capaccio, A., Centofanti, R., Neri, M., Cruciani, A.R., & Maisto, L. (1988). Intrapleural natural Beta-Interferon in the treatment of malignant pleural effusions. Oncology 45, 253-256.

Rudick, R.A., Carpenter, C.S., Cookfair, D.L., Tuohy, V.K., & Ransohoff, R.M. (1993). *In vitro* and *in vivo* inhibition of mitogen driven T-cell activation by recombinant interferon beta. Neurology 43, 2080-2087,

Ruffner, H., Reis, L.F., Naf, D., & Weissmann, C. (1993). Induction of Type I Interferon genes and Interferon-inducible genes in embryonal stem cells devoid of Interferon Regulatory Factor 1. Proc. Natl. Acad. Sci. USA 90, 11503-11507.

Rusconi, S., Agarossi, A., Ravasi, L., Tonta, A., Conti, M., & Galli, M. (1994). Serum 2'-5' oligoadenylate synthetase levels and clinical response to interferon-beta in women with genital human papillomavirus infection. J. Infect. Dis. 169, 1112-1115.

Ruzicka, F.J., Jach, M.E., & Borden, E.C. (1987). Binding of recombinant-produced Interferon-beta$_{ser}$ to human lymphoblastoid cells: Evidence of two binding domains. J. Biol. Chem. 263, 16142-16149.

Rysiecki, G., Gewert, D.R., & Williams, B.R. (1989). Constitutive expression of a 2'-5' oligoadenylate synthetase cDNA results in increased antiviral activity and growth suppression. J. Interferon Res. 9, 649-657.

Samuel, C.E. (1992). The RNA-dependent Pl/eIF-2α protein kinase. In: *Interferon: Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., Fleischmann, R., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Stanton, J., & Tyring, S.K. Eds.). The University of Texas Medical Branch at Galveston, pp. 237-249.

Sandberg, K., Matsson, P., & Alm, G.V. (1990). A distinct population of nonphagocytic and low level CD4+ null lymphocytes produce IFN-α after stimulation by herpes simplex virus infected cells. J. Immunol. 145, 1015-1020.

Sansonno, D., Cornacchiulo, V., Gatti, P., & Dammacco, F. (1992). Circulating levels and tissue distribution of intercellular adhesion molecule-1 during β-interferon therapy of hepatitis C virus-associated chronic active liver disease. Int. J. Clin. Lab. Res. 22, 100-105.

Saracco, G., Rizzetto, M., & Verme, G. (1994). Interferon in chronic hepatitis B. Antiviral Res. 24, 137-143.

Satoh, J.I., Paty, D., & Kim, S.U. (1995). Differential effects of beta and gamma Interferons on expression of Major Histocompatibility complex antigens and Intercellular Adhesion Molecule-1 in cultured fetal human astrocytes. Neurology, 45, 367-373.

Schattner, A., Merlin, G., Wallach, D., Rosenberg, H., Bino, T., Hahn, T., Levin, S., & Revel, M. (1981). Monitoring of Interferon therapy by assay of 2'-5' oligo-isoadenylate synthetase in human peripheral white blood cells. J. Interferon Res. 1, 587-594.

Schattner, A., Meshorer, A., Revel, M., & Wallach, D. (1982). Interferon as a possible cause of virus-related lymphocytopenia. Israel J. Med. Sci. 18, 965-966.

Schiller, J.H., Storer, B., Bittner, G., Willson, J.K.V., & Borden, E.C. (1988). Phase II trial of Interferon-βser and IFN-γ in patients with advanced malignant melanoma. J. Interferon Res. 8, 581-589.

Schmidt, C.A., Neubauer, A., Seeger, K.H., Rochlitz, C.F. Binder, T., Oettle, H., Henze, G., Liu, E.T., Huhn, D., & Siegert, W. (1994). Detection of allelic loss within beta 1-Interferon gene in childhood acute lymphoblastic leukemia using differential PCR. Ann. Hematol. 68, 171-174.

Schneider, A., Papendick, U., Gissman, L., & DeVilliers, E.M. (1987). Interferon treatment of human genital papillomavirus infection: Importance of viral type. Int. J. Cancer 40, 610-614.

Schonfeld, A., Nitke, S., Schattner, A., Wallach, D., Crespi, M., Hahn, T., Levavi, H., Yarden, O., Shoham, J., Doerner, T., & Revel, M. (1984). Intramuscular human interferon-β injections in treatment of condylomata acuminata. Lancet I, 1038-1042.

Sciavolino, P.J., Lee., T.H., & Vilcek, J. (1994). Interferon-β induces metalloproteinase mRNA expression in human fibroblasts: Role of AP-1. J. Biol. Chem. 269, 21627-21634.

Sehgal, P.B., & Tamm, I. (1979). Two mechanisms contribute to the superinduction of poly (I)-poly (C) induced human fibroblast interferon production. Virology 92, 240-244.

Sehgal, P.B., Dobberstein, B., & Tamm, I. (1977). Interferon message content of human fibroblasts during induction, shutoff and superinduction of Interferon production. Proc. Natl. Acad. Sci. USA 74, 3409-3413.

Selmaj, K.W., & Raines, C.S. (1988). Tumor necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. Ann. Neurol. 23, 339-345.

Semprini, A.E., Stillo, A., Marcozzi, S., Castagna, C., Fiore, S., & Radaelli, U.(1994). Treatment with interferon for genital HPV in HIV-positive and HIV-negative women. Eur. J. Obstet. Gynecol. 53, 135-137.

Sen, R., & Baltimore, D. (1986). Inducibility of κ immunoglobulin enhancer binding protein NF-κB by a posttranslational mechanism. Cell 47, 921-928.

Sen, G.S., & Lengyel, P. (1992). The Interferon system. J. Biol. Chem. 267, 5017-5020.

Senda, T., Shimazu, T., Matsuda, S., Kawano, G., Shimizu, H., Nakamura, K.T., & Mitsui, Y. (1992). Three dimensional crystal structure of recombinant murine Interferon-β. EMBO J. 11, 3193-3201.

Shafik, H.M., Nokta, M.A., & Pollard, R.B. (1991). Recombinant human Interferon-betaser protects against zidovudine-induced genetic damage in AIDS patients. Antiviral Res. 16, 205-212.

Sharief, M.K., & Thompson, E.J. (1992). *In vivo* relationship of Tumor necrosis factor-α to blood-brain barrier damage in patients with active multiple sclerosis. J. Neuroimmunol. 38, 27-34.

Shuai, K., Horvath, C.M., Huang, L.S.T., Qureshi, S.A., Cowburn, D., & Darnell, J.E. (1994). Interferon activation of the transcription factor Stat91 involves dimerization through SH-2-phosphotyrosyl peptide interactions. Cell 76, 821-828.

Shupack, J., Stiller, M., Davis, I., Kennym C., & Jondreau, L. (1992). Topical alpha-Interferon ointment with dimethyl-sulfoxide in the treatment of recurrent genital herpes simplex. Dermatology 184, 40-44.

Sica, G., Natoli, V., Stella, C., & Del Bianco, S. (1987). Effect of natural beta-Interferon on cell proliferation and steroid receptor level in human breast cancer cells. Cancer 60, 2419-2423.

Sica, G., Angelucci, C., Iacopino, F., Lama, G., Robustelli, G., Serlupi-Crescenzi, O., & Donini, S. (1992). Effect of natural beta-Interferon on estrogen receptor mRNA of breast cancer cells. Anticancer Res. 12, 2061-2064.

Sica, G., Iacopino, F., Lama, G., Amadori, D., Baroni, M., LoSardo, F., Malacarne, P., Marchetti, P., Pellegrini, A., Zaniboni, A., et al. (1993a). Steroid receptor enhancement by natural Interferon-β in advanced breast cancer. Eur. J. Cancer 29A, 329-333.

Sica, G., Iacopino, F., Lama, G., Marchetti, P., Carenza, L., Dell'Acqua, S., De Cicco Nardone, F., Lombardi, A., Mariani, L., Villani, C., et al. (1993b). Natural interferon-beta and steroid hormone receptors in primary endometrial cancer. Gynecol. Oncol. 50, 185-190.

Sica, G., Dell'Acqua, G., Iacopino, F., Fattorossi, A., Marchetti, P., vander Kwast, T.H., & Pavone-Macaluso, M. (1994). Androgen receptors and hormone sensitivity of a human prostatic cancer cell line (PC-3) are modulated by natural beta-Interferon. Urol. Res. 22, 33-38.

Sidky, Y.A., & Borden, E.C. (1987). Inhibition of angiogenesis by Interferons. Effects on tumor- and lymphocyte-induced vascular responses. Cancer Res. 47, 5155-5161.

Siegrist, C.A., & Mach, B. (1993). Antisense oligonucleotides specific for regulatory factor RFX-1 inhibit inducible but not constitutive expression of all Major Histocompatibility class II genes. Eur. J. Immunol. 23, 2903-2908.

Sims, S.H., Cha, Y., Romine, M.F., Gao, P.Q., Gottlieb, K., & Deisseroth, A.B. (1993). A novel Interferon-inducible domain: Structural and functional analysis of the human Interferon Regulatory Factor 1 gene promoter. Mol. Cell. Biol. 13, 690-702.

Soh, J., Mariano, T.M., Lim, J.K., Izotova, L., Mirochnitchenko, O., Schwartz, B., Langer, J.A., & Pestka, S. (1994). Expression of a functional human type I Interferon receptor in hamster cells: application of functional YAC screening. H. Biol. Chem. 269, 18102-18110.

Solinas, A., Cossu, P., Poddighe, P., Tocco, A., Deplano, A., Garrucciu, G., & Diana, M.S. (1993). Changes of serum 2',5' oligoadenylate synthetase activity during interferon treatment of chronic hepatitis C. Liver 13, 253-258.

Soos, J.M., & Johnson, H.M. (1995). Type I Interferon inhibition of superantigen stimulation: implications for treatment of superantigen-associated disease. J. Interferon Cyt. Res. 15, 39-45.

Stark,G.R., & Kerr, I.M. (1992). Interferon-dependent signaling pathways: DNA elements, transcription factors, mutations, and effect of viral proteins. J. Interferon Res. 12, 147-151.

Steimle, V., Siegrist, A.C., Mottet, A., Lisowska-Grospierre, B., & Mach. B. (1994). Regulation of MHC class II expression by the transactivator gene CIITA. Science 265, 106-109.

Stein, S., Kenny, C., Friesen, H.J., Shively, J., DelValle, U., & Pestka, S. (1980). NH2-terminal amino acid sequence of human fibroblast Interferon. Proc. Natl. Acad. Sci. USA 77, 5716-5719.

Stewart, W.E. (1979). Priming. In: *The Interferon System* (Stewar, W.E., Ed.). Springer-Verlag, New York, pp. 233-236.

Strander, H., & Oberg, K. (1992). Clinical use of interferons: Solid tumors. In: *Interferon, Principles and Medical Applications* (Baron, S., Coppenhaver, D.H., Dianzani, F., Fleishmann, R., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Stanton J., & Tyring, S.K. Eds). The University of Texas Medical Branch, pp. 533-561.

Straus, S.E., Seidlin, M., Takiff, H.E., Rooney, J.F., Nusinoff-Lehrman, S., Bachrach, S., Felser, J.M., DiGiovanna, J.J., Grimes, G.J., Krakauer, H., Hallahan, C., & Alling, D. (1986). Double-blind comparison of weekend and daily regimens of oral acyclovir for suppression of recurrent genital herpes. Antiviral Res. 6, 151-159.

Sugawa, N., Ekstrand, A.J., Ueda, S., & Collins, V.P. (1993). Frequency of IFN-beta-1 gene loss in 47 primary human gliomas. Noshuyo Byori 10, 161-163.

Tabor, E., & Kabayashi, K. (1992). Hepatitis C virus, a causative infectious agent of non-A, non-B hepatitis: Prevalence and structure—Summary of a conference of hepatitis C virus as a cause of hepatocellular carcinoma. J. Natl. Cancer Inst. 84,86-90.

Takano, S., Satomura, Y., & Omata. M .(1994), Effects of interferon beta on non-A, non-B acute hepatitis: A prospective randomized, controlled-dose study. Japan Acute Hepatitis cooperative study group. Gastroenterology 107, 805-811.

Talmadge, K.W., Gallati, H., Sinigaglia, F., Walz, A., & Garotta, G. (1986). Identity between human Interferon-γ and "macrophage-activating factor" produced by human T-lymphocytes. Eur. J. Immunol. 16, 1471-1477.

Tanaka, N., Kawakami, T., & Taniguchi, T. (1993). Recognition DNA sequences of Interferon Regulatory Factor 1 (IRF-1) and IRF-2, regulators of cell growth and the Interferon system. Mol. Cell. Biol. 13, 4531-4538.

Tanaka, N., Ishihara, M., Kitagawa, M., Harada, H., Kimura, T., Matsuyama, T., Lamphier, M.S., Aizawa, S., Mak, T.W., & Taniguchi, T. (1994). Cellular commitment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1. Cell 77, 829-839.

Taniguchi, T. (1989). Regulation of Interferon-β gene: Structure and function of cis-elements and trans-activating factors. J. Interferon Res. 9, 633-640.

Taniguchi, T., Mantei, N., Schwarzsteinm M., Nagata, S., Muramatsu, M., & Weismann, C. (1980a). Human leukocyte and fibroblast Interferons are structurally related. Nature 285, 547-549.

Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y., & Miramatsu, M. (1980b). The nucleotide sequence of human fibroblast interferon cDNA. Gene 10, 11-15.

Thanos, D., & Maniatis, T. (1992). The high mobility group protein HMG I(Y) is required for NF-kappa B-dependent virus induction of the human IFN-beta gene. Cell 71, 777-789.

Thanos, D., & Maniatis, T. (1995). Identification of the rel family members required for virus induction of the human beta Interferon gene. Mol. Cell. Biol. 15, 152-164.

The IFN-β Multiple Sclerosis Study Group. (1993). Interferon beta-1b is effective in relapsing-remitting Multiple Sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 43, 655-661.

The IFN-β Multiple Sclerosis Study Group, and the University of British Columbia MS/MR1 Analysis Group. (1995). Interferon-beta-1b in the treatment of Multiple Scherosis: Final outcome of the randomized controlled trial. Neurology 45, 1277-1285.

Torrisi, J., Berg, C., Bonnem, E., & Dritschilo, A. (1986). The combined use of interferon and radiotherapy in cancer management. Sem. In Oncol. 13, 78-83.

Torseth, J.W., & Merigan, T.C. (1986). Significance of local IFN-γ in recurrent herpes simplex infection. J. Infect. Dis. 153, 979-984.

Traugott, U., & Lebon, P. (1988). Demonstration of α,β and γ Interferons in active chronic multiple sclerosis lesions. Ann. NY Acad. Sci. 540, 309-311.

Turek, L.P., Byrne, J.C., Lowy, D.R., Dvoretsky, I., Friedmann, R.M., & Howley, P.M. (1982). Interferon induces morphological reversion with elimination of extrachromosomal viral genomes in bovine papillomavirus-transformed cells. Proc. Natl. Acad. Sci. USA 79, 7914-1918.

Twu, J.S., Lee, C.H., Lin, P.M., & Schloemer, R.H. (1988). Hepatitis B virus suppresses expression of human β-interferon. Proc. Natl. Acad. Sci. USA 85, 252-256.

Utsumi, J., Yamazaki, S., Hosoi, K., Kimura, S., Hanada, K., Shimazu, T., & Shimizu, H. (1987). Characterization of *E.coli* derived recombinant human Interferon-beta as compared with fibroblast human Interferon-beta. J. Biochem. (Tokyo) 101, 1199-1208.

Utsumi, J., Mizuno, Y., Hosoi, K., Okano, K., Sawada, R., Kajitani, M., Sakai, I., Naruto, M., & Shimizu, H. (1989). Characterization of four different mammalian cell-derived recombinant human Interferon-β1s: Identical polypeptides and non-identical carbohydrate moieties compared to natural ones. Eur. J. Biochem. 181, 545-552.

Utsumi, J., Kasama, K., Nagahata, T., Ogawa, E., Kagawa, Y., Matsubara, K., & Satoh, Y. (1991). Different pharmacokinetics and biological actions between fibroblast-derived natural and CHO cell-derived recombinant human Interferon-βs. J. Interferon Res. 11, Suppl. 1, S160.

Uze, G., Lutfalla, G., & Gresser, I. (1990). Genetic transfer of a functional human IFN-α Receptor into mouse cells: Cloning and expression of its cDNA. Cell 60, 225-234.

Uze, G., Lutfalla, G., & Mogensen, K.E. (1995). α and β Interferons and their receptor and their friends and relations. J. Interferon Cyt. Res. 15, 3-26.

Vaiman, D., Pietrokovsky, S., Cohen, B., Benech, P., & Chebath, J. (1990). Synergism of type I and type II Interferons in stimulating the activity of the same DNA enhancer. FEBS Letters 265, 12-16.

Van den Berg, H.W., Leahey, W.J., Lynch, M., Clarke, R., & Nelson, J. (1987). Recombinant human interferon alpha increases oestrogen receptor expression in human breast cancer cells (ZR-75-1) and sensitizes them to the antiproliferative effects of tamoxifen. Br. J. Cancer 55, 255-257.

VanDamme, J., Schaafsma, M.R., Fibbe, W.E., Falkenburg, JHF,, Opdenakker. G., & Billiau, A. (1989). Simultaneous production of Interleukin 6, Interferon β and colony-stimulating activity by fibroblasts after viral and bacterial infection. Eur. J. Immunol. 19, 163-168.

Vannini, A., Cembrano, S., Assetto, V., & Gianitelli, A. (1986.) Interferon-beta treatment of herpes simplex keratitis. Ophtalmologica 192, 6-13.

Veals, S.A., Schindler, C., Leonard, D., Fu, X.Y., Aebersold, R., Darnell, J.E., & Levy, D.E.. (1992). Subunit of an alpha-Interferon-responsive transcription factor is related to Interferon Regulatory Factor and Myb families of DNA-binding protein. Mol. Cell. Biol. 12, 3315-3324.

Velazquez, L., Fellous, M., Stark, G.R., & Pellegrini, S. (1992). A protein tyrosine kinase in the alpha/beta signaling pathway. Cell 70, 313-322.

Velazquez, L., Mogensen, K.E., Barbieri, G., Fellous, M., Uze, G., & Pellegrini, S. (1995). Distinct domains of the tyrosine kinase tyk2 required for high

affinity binding of a/b IFNs and for signal transduction. J. Biol. Chem. 270, 3327-3334.

Vial, T., & Descotes, J. (1994). Clinical toxicity of the Interferons. Drug Saf. 10, 115-150.

Viellard, V., Lauret, E., Rousseau, V., & DeMaeyer, E. (1994). Blocking of retroviral infection at a step prior to reverse transcription in cells transformed to constitutively express Interferon-beta. Proc. Natl. Acad. Sci. USA 91, 2689-2693.

Viladomiu, L., Genesca, J., Esteban, J., Allende, H., Gonzalez, A., Lopez-Talavera, J.C., Esteban, R., & Guardia, J. (1992). Interferon-α in acute post-transfusion hepatitis C: A randomized controlled trial. Hepatology 15, 767-769.

Villar, A., Massuti, B., Candel, M., Lizon, J., Frau, A., Gorostidi, B., & Ales-Martinez, J.E. (1995). Survival benefit from adding Interferon-β (Frone) to a fluorouracil regimen in advanced colorectal cancer. Abstract ASCO Annual Meeting May 20-23, 1995.

Visvanathan, K.V., & Goodburn, S. (1989). Double-stranded RNA activates binding of NF-κB to an inducible element in human β-Interferon promoter. EMBO J. 8, 1129-1138.

Von Wild, K.R., & Knocke, T.H. (1991). The effect of local and systemic interferon-beta (Fiblaferon) on supratentorial malignant cerebral glioma: A phase II sudy. Neurosurg. Rev. 14, 203-213.

VonWussow, P., & Borden, E.C. (1989). Antibodies to Interferons in patients: defining their significance. J. Interferon Res. 9, special supplement.

Vuoristo, M., Jantunen, I., Pyrhonen, S., Mulhonen, T., & Kellokumpu-Lehtinen, P. (1994). A combination of subcutaneous recombinant interleukin-2 and recombinant interferon-alpha in the treatment of advanced renal cell carcinoma or melanoma. Eur. J. Cancer 30A, 530-532.

Wadler, S., & Wiernick, P.H. (1990). Clinical update on the role of fluorouracil and recombinant interferon alfa-2a in the treatment of colorectal carcinoma. Semin. In Oncology 17, (Suppl 1), 16-21.

Wakabayashi, T., Yoshida, J., Mizuno, M., Kitto, A., & Sugita, K. (1992). Effectiveness of interferon-beta, ACNU and radiation therapy in pediatric patients with brainstem glioma. Neurol. Med. Chir. 32, 942-946.

Watanabe, N., Sakakibara, J., Hovanessian, A.G., Taniguchi, T., & Fujita, T. (1991). Activation of IFN-β element by IRF-1 requires a post-translational event in addition to IRF-1 synthesis. Nucleic Acids Res. 19, 4421-4428.

Watling, D., Pellegrini, S., Stark, G.R., & Kerr. I.M. (1992). A human cell line responsive to beta but not alpha Interferons. J. Interferon Res. 12 (Suppl. 1), S91.

Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L.M., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P., & Revel, M. (1980). Two interferon mRNAs in human fibroblasts: *In vitro* translation and *Escherichi coli* cloning studies. Proc. Natl. Acad. Sci. USA 77, 7152-7156.

Weissmann, C., & Weber, H. (1986). The Interferon genes. In: *Progress in Nucleic Acids Research and Molecular Biology,* Vol. 33 (Cohn, W.E., & Kivic, M., Eds.). Academic Press, New York, pp. 251-302.

Weisz, A., Marx, P., Sharf, R., Appella, E., Driggers, P.H., Ozato, K., & Levi, B-Z. (1992). Human Interferon consensus sequence binding protein is a negative regulator of enhancer elements common to Interferon-inducible genes. J. Biol. Chem. 267, 25589- 25596.

Welander, C.E., Homesley, H.D., Smiles, K.A., & Peets, E.A. (1990). Intralesional interferon alfa-2b for the treatment of genital warts. Am. J. Obstet. Gynecol. 162, 348-354.

Wells, V., & Mallucci, L. (1988). Cell cycle regulation (G1) by autocrine Interferon and dissociation between autocrine Interferon and 2'-5' oligoadenylate synthetase expression. J. Interferon Res. 8, 793-802.

Whitley, M.Z., Thanos, D., Read, M.A., Maniatis, T., & Collins, T. (1994). A striking similarity in the organization of the E-selectin and beta-Interferon gene promoters. Mol. Cell. Biol. 14, 6464-6475.

Whittemore, L.A., & Maniatis, T. (1990). Post-induction turn-off of beta-interferon gene expression. Mol. Cell. Biol. 10, 1329-1337.

Wiernick, P.H., Schwartz, B., Dutcher, J.P., Turman, N., & Adinolfi, C. (1990). Successful treatment of hairy cell leukemia with beta-ser Interferon. Am. J. Hematol. 33, 244-248.

Williams, A.J., & Colby, B. (1989). Recombinant human Interferon-beta suppresses the replication of HIV and acts synergically with AZT. J. Interferon Res. 9, 709-718.

Wiranowska, M., Gonzalvo. N., Saporta, S., Gonzales, O.R., & Prockop, L.D. (1992). Evaluation of blood-brain barrier permeabiliy and the effect of interferon in mouse glioma model. J. Neurooncol 14, 225-236.

Wiranowska, M., Prockop, L.D., Naidu, A.K., Saporta, S., Kori, S., & Kulkarni, A.P. (1994). Interferon entry through the blood-brain barrier in glioma and its effect on lipoxygenase activity. Anticancer Res. 14, 1121-1126.

Witt, P.L., Goldstein, D., Storer, B.E., Grossberg, S.E., Flashner, M., Colby, C.B., & Borden, E.C. (1992). Absence of biological effects of orally administered Interferon-βser. J. Interferon Res. 12, 411-413.

Witt, P.L., Storer, B.E., Bryan, G.T., Brown, R.R., Flashner, M., Larocca, A.T., Colby, C.B., & Borden, E.C. (1993). Pharmacodynamics of biological response *in vivo* after single and multiple doses of Interferon-β. J. Immunother. 13, 191-200.

Wucherpfennig, K.W., Weiner, H.L., & Hafler, D.A. (1991). T-cell recognition of myelin basic protein. Immunol. Today 12, 277-282.

Yagi, K., Hayashi, Y., Ishida, N., Ohbayashi, M., Ohishi, M., Ohishi, N., Mizuno, M., & Yoshida, J. (1994). Interferon-beta endogenously produced by intratumoral injection of cationic liposome-encapsulated gene: Cytocidal effect on glioma transplanted into nude mouse brain. Biochem. Mol. Biol. Int. 32, 167-171.

Yang, Y.L., Reis, L.F.L., Pavlovic, J., Aguzzi, A., Schafer, R., Kumar, A., Williams, B.R.G., Aguet, M., & Weissmann, C. (1995). Deficient signaling in mice devoid of double-stranded RNA-dependent protein kinase. EMBO J. 14, 6095-6106.

Yarden, A., Shure-Gottlieb, H., Chebath, J., Revel, M., & Kimchi, A. (1984). Autogenous production of IFN-β switches-on HLA genes during differentiation of histiocytic lymphoma U937 cells. EMBO J. 3, 969-973.

Yaron, M., Yaron, I., Gurari-Rotman, D., Lindner, HR., & Zor, U. (1977). Stimulation of prostaglandin E production in cultured human fibroblasts by poly rI:rC and human Interferon. Nature 267, 457-459.

Yliskoski, M., Syrjanen, S., Syrjanen, S., Saarikoski, S., & Nethershell, A. (1991). Systemic α-interferon (Wellferon) treatment of genital human papillomavirus (HPV) type 6, 11, 16 and 1 infections: Double-blind, Placebo-controlled trial. Gynecol. Oncol. 43, 55-60.

Yoshida, J., Kajita, Y., Wakabayashi, T., & Sugita, K. (1994). Long-term follow-up results of 175 patients with malignant glioma: Importance of radical tumor resection and postoperative adjuvant therapy with interferon, ACNU and radiation. Acta Neurochir. 127, 55-59.

Yung, W.K., Prados, M., Levin, V.A., Fetell, MR., Benett, J., Mahaley, M.S., Salcman, M., & Etcubanas, E. (1991). Intravenous recombinant interferon-beta in patients with recurrent malignant gliomas: A phase I/II study. J. Clin. Oncol. 9, 1945-1949.

Zhang, X., Alley, E.W., Russell, S.W., & Morrison, D.C. (1994). Necessity and sufficiency of beta Interferon for nitric oxide production in mouse peritoneal macrophages. Infect. Immun. 62, 33-40.

Zinn, K., Keller, A., Whittemore, L.A., & Maniatis, T. (1988). 2-aminopurine selectively inhibits the induction of beta-Interferon, c-fos and c-myc gene expression by viruses and poly rI:rC. Science 240, 210-213.

THE MOLECULAR BASIS OF IFNγ ACTION

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I. INTRODUCTION

Interferon-γ (IFN-γ) belongs to a family of proteins that are related by their ability to protect cells from viral infection. Based on several criteria the interferons have been divided into three distinct classes termed interferon-α, -β, and -γ (Table 1). IFNα (originally known as Type I IFN or Leukocyte IFN because it was produced by peripheral blood mononuclear cells) and IFNβ (also originally known as Type I IFN or Fibroblast IFN because of its cell of origin) are classical interferons induced in response to viral infection of cells (Pestka et al., 1987; Stewart, 1979). IFNγ (also termed Type II IFN or Immune IFN) is unrelated to the Type I interferons at both the genetic and the protein levels (Gray et al., 1982; Gray and Goeddel, 1982, 1983). Moreover, IFNγ is induced by a unique set of stimuli and is produced only by T lymphocytes and natural killer (NK) cells. Importantly, viral infection of these cells does not directly induce IFNγ production. Although IFNγ displays most of the biologic activities that have been ascribed to the other interferons, it has a 10-100-fold lower specific antiviral activity than either IFNα or IFNβ. On the other hand, IFNγ is 100-10,000 times more active as an immunomodulator compared to the other classes of interferons (Pace et al., 1985). This observation has lead to the concept that whereas IFNα/β are primarily antiviral agents which display some immunomodulatory activity, IFNγ is primarily an immunomodulator that also can exert some antiviral activity (De Maeyer, 1984).

This chapter will emphasize the advances that have occurred over the past 10 years which have significantly enhanced our understanding of IFNγ biology and biochemistry. Moreover, this review will delineate the recent findings that have led to a comprehensive understanding of the molecular events that underlie IFNγ's pleotropic effects cells.

Table 1. Properties of the Interferons

Property	*IFNα*	*IFNβ*	*IFNγ*
Nomenclature	Type I Leukocyte	Type I Fibroblast	Type II Immune
Major Inducers	Virus	Virus, LPS ds-poly RNA	Antigens Mitogens
Physical Properties			
M.W. (kDa)			
Predicted/mature	20/20	20/20-25	17/34-50
Amino acids	165-166	166	143
Subunite composition	Single polypeptide	Single polypeptide	Nonvonvalent homodimer
Gene Structure			
Number of genes	26	1	1
Chromosomal location			
Murine	4	4	10
Human	9	9	12
Cellular Source	T cells, B cells, and macrophages	Fibroblasts and epithelial cells	T cells and NK cells

II. MOLECULAR CHARACTERISTICS OF HUMAN AND MURINE IFNγ

The cDNAs for human and murine IFNγ were first cloned in 1982 by Gray and Goeddel (Gray et al., 1982; Gray and Goeddel, 1982, 1983). Today a great deal is known about the structure of the IFNγ genes and the corresponding proteins they encode. In both humans and mice there is only a single IFNγ gene. This gene is considerably more complex than the genes for either IFNα or IFNβ. The human and murine IFNγ genes are 6 kb in size and each contains four exons and three introns. Using *in situ* hybridization techniques, the genes for human and murine IFNγ have been localized to chromosomes 12 (12q24.1) and 10, respectively (Trent et al., 1982; Naylor et al., 1983, 1984)

Activation of the human gene leads to the generation of a 1.2 kb mRNA that encodes a 166 amino acid polypeptide (Table 2; Gray et al., 1982; Derynck et al., 1982). The amino-terminal 23 residues of the

Table 2. Comparison of Human and Murine IFNγ

Human	MKYTSYILAF	QLCIVLGSLG	CYCQDPYVKE	AENLKKYFNA	GHSDVADNGT	50
	* * ****	** * *	***	* * ***	**	
Murine	MNATHCILAL	QLFLMAVS-G	CYCHGTVIES	LESLNNYFNS	SGIDVEEK-S	48
Human	LFLGILKNWK	EESDRKIMQS	QIVSFYFKLF	KNFKDDQSIQ	KSVETIKEDM	100
	*** * **	* ** **	** *** **	** * *	*	
Murine	LFLDIWRNWQ	KDGDMKILQS	QIISFYLRLF	EVLKDNQAIS	NNISVIESHL	98
Human	NVKFFNSNKK	KRDDFEKLTN	YSVTDLNVQR	KAIHELIQVM	AELSPAAKTG	150
	** *	* * *	* ***	* *** *	* *	
Murine	ITTFFSNSKA	KKDAFMSIAK	FEVNNPQVQR	QAFNELIRVV	HQLLPESSLR	148
Human	KRKRSQMLFQ	GRRASQ	166			
	*****					
Murine	KRKRSRC--	——	155			

human protein constitute a typical hydrophobic signal sequence which, when proteolytically removed, gives rise to a mature 143 residue, positively charged polypeptide with a predicted molecular mass of 17 kDa. The natural mature IFNγ polypeptide contains N-linked carbohydrates and displays an apparent Mr of 25 kDa. Two natural polypeptides self-associate non-covalently to form a homodimer with an apparent molecular mass of 50 kDa (Scahill et al., 1983; Le et al., 1985; Chang et al., 1984; Arakawa et al., 1986; Yphantis and Arakawa, 1987; Nagata et al., 1987). At physiologic concentrations, little, if any, monomer is detectable. Only the dimer can display IFNγ biologic activity because it is the only form of the molecule that can effect IFNγ receptor dimerization (Greenlund et al., 1993; Dighe et al., 1993).

The murine gene gives rise to a 1.2 kb mRNA that encodes a mature 134 amino acid polypeptide with a predicted molecular mass of 15.4 kDa (Table 2; Gray and Goeddel, 1983). Like its human counterpart, murine IFNγ exists exclusively as a noncovalent homodimer. Human and murine IFNγ display only modest identity at either the cDNA or amino acid levels (60% and 40%, respectively). This low level of sequence identity explains why the human and murine proteins display a strict species specificity in their ability to bind to and activate human and murine cells.

Recently, the x-ray crystallographic structure of human IFNγ was solved to 3.5 Å (Ealick et al., 1991). This study confirmed the dimeric nature of the mature protein. The individual subunits have a flattened prolate elliptical shape. However, the overall structure of the dimer is

compact and globular. The molecule appears to be primarily helical (62%) and lacks β sheet structure. Each subunit consists of six α helices held together by short nonhelical regions. The dimer is formed by a unique intertwining of the helices across the subunit face which provides an opportunity for multiple interactions along each subunit. This type of intimate subunit interaction is extremely unusual and has been seen only in a few other proteins. The model predicts that the subunits associate in an antiparallel fashion, thereby leading to a juxtaposition of the amino and carboxy terminal portions of the opposing polypeptide chains. This structure suggests that each IFNγ dimer may be able to bind two IFNγ receptors. Recently obtained experimental data support this possibility (Greenlund et al., 1993; Dighe et al., 1993).

III. IFNγ BIOSYNTHESIS

In the normal host, T lymphocytes represent a major cellular source of IFNγ (Figure 1). All $CD8^+$ T cell populations and certain subsets of $CD4^+$ T cells can produce the protein (Vilĉek et al., 1985; Schreiber and Celada, 1985). IFNγ synthesis has been demonstrated in the Th1 helper T cell subset and by the less differentiated/activated type of $CD4^+$ T cell designated Th0 (Street et al., 1990; Gajewski et al., 1989a; Trinchieri and Perussia, 1985). The external stimuli that induce IFNγ production by T cells are similar to those that induce other T cell-derived cytokines (Vilcek et al., 1985; Schreiber and Celada, 1985; Trinchieri and Perussia, 1985). The primary physiologic stimulus is antigen in the context of either major histocompatibility (MHC) Class II (for $CD4^+$ T cells) or MHC Class I proteins (for $CD8^+$ T cells). Experimentally, IFNγ can also be induced by either (1) direct antibody stimulation of the T cell receptor/CD3 complex, (2) T cell mitogens (such as concanavalin A or phytohemagglutinin), or (3) pharmacologic stimuli (such as the combination of phorbol myristate acetate and calcium ionophore; Gajewski et al., 1989b). Stimulation of T cells results in the induction of IFNγ mRNA which is first detectable at 6-8 h, peaks by 12-24 h and slowly declines thereafter. The protein is secreted immediately after synthesis. It can first be detected in the extracellular environment 8-12 h, after stimulation and reaches peak levels after 18-24 h. IFNγ produced as a result of experimental, *in vitro* T cell stimulation (such as during the mixed leukocyte reaction) is not significantly consumed by the cells of the culture and can therefore be detected in the medium long after the T cell activation

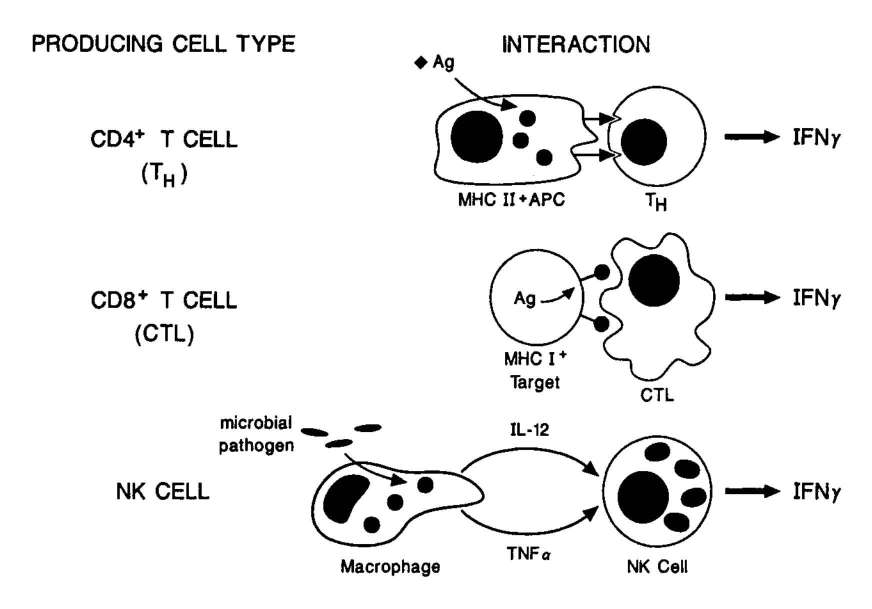


Figure 1. Cellular sources of IFNγ. IFNγ can be produced either by $CD4^+$ T cells in response to antigen presented in the context of MHC class II molecules or by cytotoxic T lymphocytes following recognition of antigen associated with MHC class I. In addition, NK cells elaborate IFNγ after exposure to TNFα andIL-12.

response has ended (Schreiber et al., 1983). In contrast, IFNγ is rarely seen in the circulation of humans or mice undergoing immunologic stimulation. This apparent discrepancy between *in vitro* and *in vivo* levels of fluid phase IFNγ is most likely due to the rapid removal of IFNγ from the circulation by IFNγ receptors that are ubiquitously expressed on nearly all cells (Langer and Pestka, 1988).

Two newly described cytokines are noteworthy in their respective abilities to regulate IFNγ production in either a positive or negative manner. IL-12 (formerly called NK stimulatory factor, NKSF) is a product of B cells and macrophages and induces IFNγ gene expression in T cells and NK cells in a manner that is at least partially distinct from the conventional pathway of T cell activation (Wolf et al., 1991; Stern et al., 1990). IL-12 dependent IFNγ induction is insensitive to cyclosporin A and is synergistic with phytohemagglutinin, phorbol esters, anti-CD3, IL-2, and allogeneic antigens but not Ca^{2+} ionophores (Chan et al., 1992). IL-12 is induced in macrophages by infection with bacteria or interaction with bacterial products such as LPS. Another newly

described cytokine, IL-10, is an important *inhibitor* of IFNγ production by both NK and T cells (Moore et al., 1993; Tripp et al., 1993). In both cases, IL-10 exerts its actions indirectly on the accessory cell populations and not on the cell that produces IFNγ. For NK cells, IL-10 prevents IFNγ production by inhibiting TNF and IL-12 production by macrophages thereby preventing the generation of the agents responsible for stimulating NK cells. For T cells, the effect of IL-10 has also been traced to the macrophage antigen presenting cells and appears to be due to the inhibition of macrophage derived accessory molecules required to induce full activation of the T cell for IFNγ production. At least one of these factors is IL-12.

More recent studies have demonstrated that IFNγ can also be produced by natural killer cells (NK; Figure 1; Handa et al., 1983; Bancroft et al., 1987, 1989, 1992; Wherry et al., 1991). The pathway leading to IFNγ production by NK cells is not MHC restricted nor contact-mediated and has recently been elucidated. Bacterial or microbial products stimulate macrophages to produce TNFα and IL-12 (Bancroft et al., 1991; Tripp et al., 1993). Together these two cytokines then stimulate NK cells to produce IFNγ. Since antigen specific cells are not required in this reaction, NK cell-derived IFNγ is produced within hours after infection leading to a rapid activation of surrounding macrophage populations. The physiologic importance of this pathway is derived from two facts. First, it provides the host with a first line of defense that can keep a microbial infection in check until such time that T cell-dependent sterilizing immunity can be generated. Second, the presence of IFNγ in the milieu in which specific T cell populations are being induced helps to establish the specific types of T cell subsets that are produced and consequently determines whether cellular or humoral immune responses eventually develop.

IV. THE IFNγ RECEPTOR

IFNγ mediates its pleiotropic immunomodulatory effects through binding, in a species specific manner, to a high affinity ($K_A = 10^9$-10^{10} M^{-1}) cell surface receptor that is expressed on nearly all cell surfaces (Farrar and Schreiber, 1993; Anderson et al., 1982; Finbloom et al., 1985; Littman et al., 1985; Ucer et al., 1986; Novick et al., 1987; Aguet and Merlin, 1987; Calderon et al., 1988; Basu et al., 1988; Stefanos et al., 1989; Fountoulakis et al., 1989; Van Loon et al., 1991). Over the past

several years, the structure and function of the IFNγ receptor polypeptide subunits have been defined, and their cDNAs cloned. Functionally active IFNγ receptors are now known to consist of two species of matched polypeptides: the IFNγ receptor α chain which plays the predominant role in ligand binding and ligand trafficking through the cell and which is necessary but not sufficient for signaling and the IFNγ receptor β chain or Accessory Factor-1 (AF-1) needed predominantly for signaling (Farrar and Schreiber, 1993; Schreiber and Aguet, 1994).

A. The IFNγ Receptor α Chain

The cDNA encoding the ligand binding subunit (α chain) of the human IFNγ receptor (CDw119) cDNA was isolated in 1988 and encodes a 489 amino acid precursor that contains a 17 amino acid signal sequence (Table 3 and Figure 2; Aguet et al., 1988). Although the mature, nonglycosylated 472 amino acid polypeptide has a predicted molecular mass of 52.5 kDa, it displays an M_r of 65 kDa when analyzed by SDS-PAGE (Farrar and Schreiber, 1993). The molecule is symmetrically oriented around a single 23 amino acid transmembrane domain. The extracellular domain consists of 228 amino acids and includes 10 cysteine residues and five potential N-linked glycosylation sites. In the fully mature molecule, all five sites are occupied by carbohydrate which contribute 20-35 kDa to the apparent molecular mass of the protein (Hershey and Schreiber, 1989; Mao et al., 1989; Fischer et al., 1990b). IFNγ receptor α subunits from different cells display M_r that vary between 80-95 kDa due to cell-specific differences in glycosylation (Hershey and Schreiber, 1989; Mao et al., 1989; Fischer et al., 1990b). The intracellular domain, which is devoid of intrinsic kinase or phosphatase activities, consists of 220 amino acids, and contains five tyrosine residues and a high percentage (24%) of serine and threonine residues. Based on the general structural features of the extracellular domain, the IFNγ receptor α chain has been classified as a member of the Type II Cytokine Receptor Family that also includes the receptors for IFNα/β, IL-10, and tissue factor (Bazan, 1990; Ho et al., 1993). The gene encoding the human α chain is located on chromosome 6 (Jung et al., 1987; Pfizenmaier et al., 1988).

The murine IFNγ receptor α subunit gene (Gray et al., 1989; Kumar et al., 1989; Munro and Maniatis, 1989; Hemmi et al., 1989; Cofano et al., 1990) has been localized to chromosome 10 and encodes a 477 amino

Table 3. Comparison of Human and Murine IFNγ Receptor α Chains

Human	MA——-L LFLLPLVMQG -VSRAEM-GT ADLGPSSVPT PTNVTIESYN	41
	* * * * * * * *** **** * ***	
Murine	MGPQAAAGRM ILLVVLMLSA KVGSGALTST EDPEPPSVPV PTNVLIKSYN	20
Human	MNPIVYWEYQ IMPQVPVFTV EVKNYGVKNS EWIDACINIS HHYCNISDHV	91
	** * **** * * * *** ** * * * * *** * ***	
Murine	LNPVVCWEYQ NMSQTPIFTV QVKVY--SG SWTDSCTNIS DHCCNIYGQI	97
Human	GDPSNSLWVR VKARVGQKES AYAKSEEFAV CRDGKIGPPK LDIRK-EEKQ	140
	* * * * *** ****** ** * ** * ** *** * ** * *	
Murine	MYPDVSAWAR VKAKVGQKES DYARSKEFLM CLKGKVGPPG LEIRRKKEEQ	147
Human	IMIDIFHPSV FVNGDEQEVD YDPETTCYIR VYNVYV-RMN GSEIQYKILT	189
	*** * *** * *** * *** ** *	
Murine	LSVLVFHPEV VVNGESQGTM FGDGSTCYTF DYTVYVEHNR SGEILHTKHT	197
Human	QKEDDCDEIQ CQLAIPVSSL NSQYCVSAEG VLHVWGVTTE KSKEVCITIF	239
	* * * * * * ** * * ** * * * * ** *** *** *	
Murine	VEKEECNETL CELNISVSTL DSRYCISVDG ISSFWQVRTE KSKDVCIPPF	247
Human	NSSIKGSLWI PVVAALLLFL VLSLVFICFY IKKINPLKEK SIILPKSLIS	289
	* * ** *** * * * *** * ** * * * ** ***** *	
Murine	HDDRKDSIWI LVVAPLTVFT VVILVFAYWY TKK-NSFKRK SIMLPKSLLS	296
Human	VVRSATLETK PESKYVSLIT SYQPFSLEKE VVCEEPLSPA TVPGMHTEDN	339
	** ******* ***** * ****** * * *	
Murine	VVKSATLETK PESKYSLVTP HQPAVLESET VICEEPLSTV TAP——-DS	341
Human	PGKVEHTEEL SSITEVVTTE ENIPDVVPGS HLTPIERESS SPLSSNQSEP	389
	* * *** * * * * ** * * * ****** *	
Murine	PEAAEQ-EEL SKETKALEAG GSTSAMTPDS PPTPTQRRSF SKKSSBQSGO	390
Human	GSIALNSYHS RNCSESDHSR NGFDTDSSCL ESHSSLSDSE FPPNNKGEIK	439
	* *** ** * * * * ** ** * *** * *	
Murine	CSLT–AYHS RNGSDS—— -GL——V GSGSSISDLE SLPNNNSETK	427
Human	TEGQELITVI KAPTSFGYDK PHVLVDLLVD DSGKESLIGY RRTEDSKEFS	489
	* *** ******* *** *** ***** ** * * * *	489
Murine	MAEHDPPPVR KAPMASGYDK PHMLVDVLVD VGGKESLMGY RLTGEAQELS	477

acid polypeptide containing a 26 amino acid signal peptide (Table 3). The mature 451 amino acid protein shows only 52.5% amino acid sequence identity with its human counterpart. Nevertheless, the murine and human proteins are organized in a similar manner. Both contain identically sized extracellular and transmembrane domains and relatively large serine and threonine rich intracellular domains.

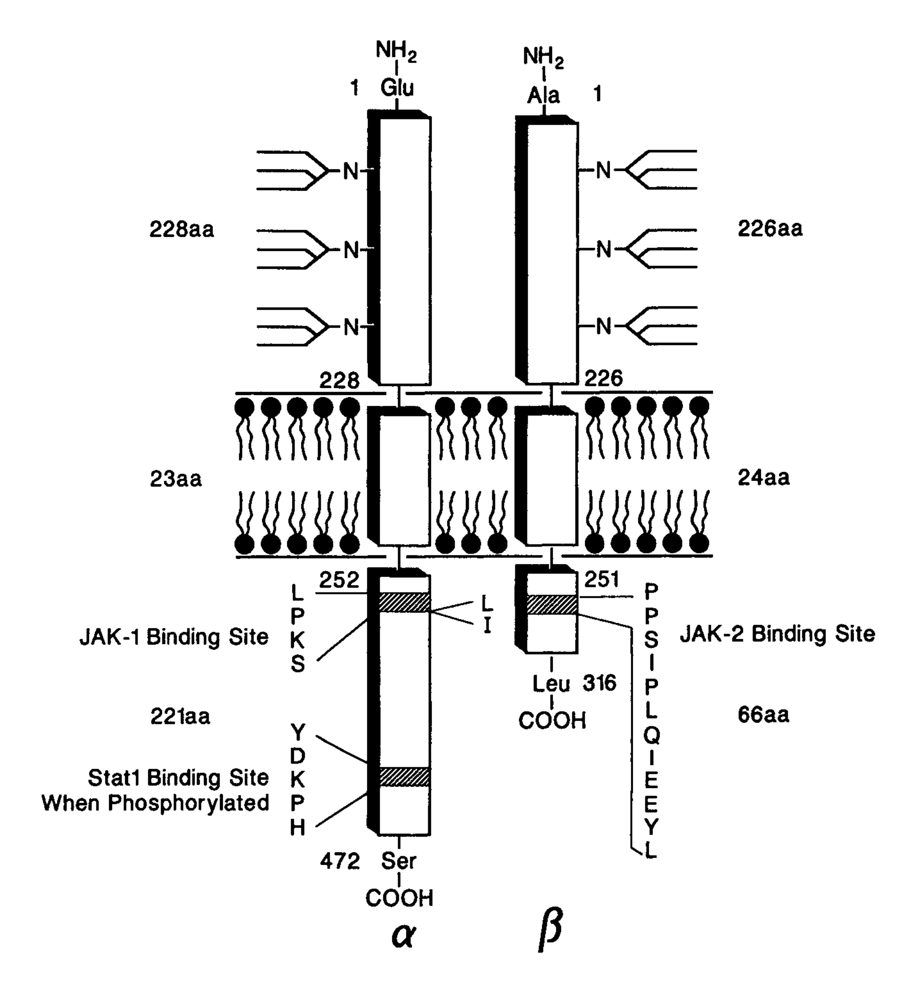


Figure 2. Polypeptide chain structure of the human IFNγ receptor.

B. The IFNγ Receptor β Chain

The human and murine IFNγ receptor β chains (or AF-1) have recently been characterized at the molecular level (Soh et al., 1994; Hemmi et al., 1994). The human β subunit is a 337 amino acid type I transmembrane polypeptide which contains a 21 amino acid signal sequence, an extracellular domain of 226 amino acids, a single 24 amino acid transmembrane domain and a relatively short intracellular domain of only 66 amino acids (Table 4 and Figure 2). The mature 316 amino

***Table 4*.** Comparison of Human and Murine IFNγ Receptor β Chains

Human	IMRPTLLWSL LLLLGVFAAA AAAPPDPLSQ LPAPQHPKIR LYNAEQVLSW	50
	*** ** *** * ** ** ** * ** * *** ** * *	
Murine	-MRPLPLWLP SLLLCGL-GA AASSPDSFSQ LAAPLNPRLH LYNDEQILTW	48
Human	EPVALSNSTR PVVYQVQFKY TDSKWFTADI MSIGVNCTQI TATECDFTAA	100
	** ** * ****** * * *** * * * ** *	
Murine	EPSPSSNDPR PVVYQVEYSF IDGSW--HR L-LEPNCTDI TETKCDLTGG	94
Human	SPSAGFPMDF NVTLRLRAEL GALHSAWVTM PWFQHYRNVT VGPPENIEVT	150
	** * * ** * * * * ** **** *** **** ** **	
Murine	GRLKLFPHPF TVFLRVRAKR GNLTSKWVGL EPFQHYENVT VGPPKNISVT	144
Human	PGEGSLIIRF SSPFDIADTS TAFFCYYVHY WEKGGIQ-Q VKGPFRSNSI	198
	** *** * * *** * *** *** * * * *** ****	
Murine	PGKGSLVIHF SPPFDVFHGA TFQY-LVHY WEKSETQQEQ VEGPFKSNSI	192
Human	SLDNLKPSRV YCLQVQAQLL WNKSNIFRVG HLSNISCYDT MADASTELQQ	248
	* **** ** **** *** * * *** ** * * ** ***	
Murine	VLGNLKPYRV YCLQTEAQLI LKNKKIRPHG LLSNVSCHET TANASARLQQ	242
Human	VILISVGTFS LLSVLAGACF FLVLKYRGLI KYWFHTPPSI PLQIEEYLKD	298
	**** * * ** * **** * *** **** ** * * ********	
Murine	VILIPLGIFA LLLGLTGACF TLFLKYQSRV KYWFQAPPNI PEQIEEYLKD	292
Human	PTQPILEALD KDSSPKDDVW DSVSIISFPE KEQEDVLQTL	338
	* * *** ** ** *** * * ******* ** ** *****	
Murine	PDQFILEVLD KDGSPKEDSW DSVSIISSPE KERDDVLQTP	332

acid protein contains five cysteines and six putative N-glycosylation sites in its extracellular domain. Like the α subunit, the β chain is also a member of the Type II Cytokine Receptor Family. The gene encoding the human receptor β chain is located on chromosome 21 (Jung et al., 1987).

The murine receptor β chain consists of an 18 amino acid signal sequence, a 224 amino acid extracellular domain, a 24 amino acid transmembrane domain, and a 64 amino acid intracellular domain (Hemmi et al., 1994). The murine β chain extracellular domain contains four cysteines, which are found in positions homologous to those in the human β chain, and six putative N-glycosylation sites. Although the entire human and murine β chain of polypeptides display 58% homology, they share 73% identity within their cytoplasmic domains. The human and murine cDNAs encode polypeptides of 38 kDa. However, mature forms of both human and murine receptor β chains display molecular masses of 62 kDa when analyzed by SDS-PAGE (Bach et al., 1996). This

difference is most likely explained by post synthetic glycosylation of the polypeptides although the composition and location of β chain-associated carbohydrates has not yet been established.

C. Structure-Function Analysis of the IFNγ Receptor Polypeptides

By expressing the receptor polypeptides either alone or in combination in cells from heterologous species, it has been possible to obtain insights into their respective functions (Jung et al., 1990; Farrar et al., 1991, 1992b; Fischer et al., 1990a; Hibino et al., 1991; Cook et al., 1992). Structure-function analysis on the IFNγ receptor subunits have now been completed resulting in the identification of the specific functionally important sequences within each receptor polypeptide (Farrar et al., 1991, 1992a, 1992b; Cook et al., 1992; Greenlund et al., 1994; Kaplan et al., 1996; Bach et al., 1996)

The specific functionally important regions within the IFNγ receptor α chain intracellular domain were identified using a combination of deletion and substitution mutagenesis approaches (Farrar et al., 1991, 1992b; Cook et al., 1992; Greenlund et al., 1994). These studies identified three topographically distinct intracellular domain sequences that are responsible for mediating distinct receptor functions (Farrar et al., 1991). One is an LI sequence residing at positions 270-271 in the human receptor α chain that is involved in effecting receptor-mediated ligand internalization/degradation (Farrar et al., 1992a; Figure 2). Disruption of this sequence either by removal or alanine substitution produced a mutant receptor α chain that showed severe defects in internalizing bound ligand and which accumulated at the cell surface. However, LI receptor mutants were able to induce IFNγ-dependent cellular responses. This result serves to dissociate the ligand trafficking and signaling roles of this receptor subunit.

A second sequence, $L_{266}PKS_{269}$ was identified as critical for induction of all IFNγ-dependent biologic responses (Greenlund et al., 1994). Substitution of single amino acids with alanine (alanine scanning) across this sequence demonstrated that only the proline residue at position 267 is required for biologic responsiveness to IFNγ (Kaplan et al., 1996). Recent studies have shown that this sequence is critical in forming a binding site on the receptor for a tyrosine kinase, known as JAK-1. JAK-1 has been shown to be required for IFNγ signaling in cells (Müller et al., 1993). In unstimulated cells, an inactive form of JAK-1 constitutively associates with the receptor α chain. Association is dependent on

the presence of an intact $L_{266}PKS_{269}$ sequence (Kaplan et al., 1996). In IFNγ-treated cells, the α chain although associated JAK-1 protein is in an activated form. Thus, although JAK-1 constitutively associates with the IFNγ receptor α chain its activation is ligand dependent.

A third functionally critical region of the receptor α chain is a $Y_{440}DKPH_{444}$ sequence, residing near the carboxy terminus of this subunit (Figure 2; Farrar et al., 1992b). Within this sequence only three residues are obligatorily required for receptor function: the tyrosine at position 440 (Y_{440}), aspartic acid at position 441 (D_{441}), and histidine at position 444 (H_{444}). Alteration of any one of these residues to alanine produced a receptor which was unable to induce IFNγ-dependent biological responses in cells (Farrar et al., 1992b). The particular functional importance of Y_{440} was confirmed by two additional observations. First, substitution of phenylalanine for Y_{440} also resulted in generation of a functionally inactive receptor (Farrar et al., 1992b). Second, mutation or deletion of any of the other tyrosine residues within the receptor's intracellular domain did not ablate receptor activity (Greenlund et al., 1994, 1995). Thus, within the entire 221 amino acid intracellular domain of the receptor α chain, only four residues (P_{267}, Y_{440}, D_{441}, and H_{444}) are required for IFNγ signal transduction.

V. SIGNAL TRANSDUCTION THROUGH THE IFNγ RECEPTOR

Recent studies have shown that following exposure of cells to IFNγ, Y_{440} becomes transiently phosphorylated (Greenlund et al., 1994; Igarashi et al., 1994). As a result of this phosphorylation, a specific binding site is created on the receptor α chain intracellular domain for an SH2 domain containing latent cytosolic transcription factor known as p91 or Stat1α. Stat1α is a member of a family of latent cytosolic transcription factors which play key roles in mediating the biologic effects of a variety of different cytokines (Darnell et al., 1994). These factors are activated by phosphorylation of key c-terminal tyrosine residues, form reciprocal dimers mediated via their SH2 domains, and translocate to the nucleus where they bind to the promoters of genes which are specifically induced by particular cytokines (Darnell et al., 1994). Work by the Darnell group has established that Stat1 is the specific STAT family member activated by IFNγ (Schindler et al., 1992; Shuai et al., 1992). The IFNγ receptor

amino acids that form the Stat1 docking site on the receptor α chain are a phosphorylated form of Y440, D441, and H444, the same residues which are obligatorily required for receptor function. Thus, ligand-dependent tyrosine phosphorylation of the IFNγ receptor α chain is the event that links receptor ligation to the signal transduction process.

A similar structure-function mutagenesis analysis has recently been performed on the human IFNγ receptor β chain (Bach et al., 1996). This study shows that, like the receptor α chain, only a fraction of the β chain intracellular domain is required for IFNγ signaling. Mutagenesis analysis revealed that the key functional residues within the receptor β chain intracellular domain are two closely juxtaposed sequences P263PSIP267 and I270EEYL274 located 13 residues away from the membrane (Bach et al., 1996). Recent work by several groups has shown that the IFNγ receptor β chain constitutively associates with the tyrosine kinase JAK-2 (Sakatsume et al., 1995; Kotenko et al., 1995; Bach et al., 1996). Like JAK-1, JAK-2 has also been shown to be required for IFNγ signaling (Watling et al., 1993). The association of JAK-2 with the IFNγ receptor β chain is mediated via the functionally critical PPSIP and IEEYL β chain sequences (Bach et al., 1996). These sequences are homologous to Box 1/Box 2 motifs which have been identified in the intracellular domains of many cytokine receptors and are thought to mediate the association of the receptors with various JAK family members (Murakami et al., 1991; Miura et al., 1993).

A. Ligand-induced Assembly and Activation of the IFNγ Receptor

IFNγ has been shown to induce dimerization of the receptor α chain both in solution and on cells (Fountoulakis et al., 1992; Greenlund et al., 1993). These studies predicted that the receptor:ligand complex formed under physiologic conditions at the cell surface consists of two receptor α chains bound to one IFNγ homodimer. This model has recently been validated by solving the crystal structure of the IFNγ receptor α chain-IFNγ complex (Walter et al., 1995). The physiologic relevance of ligand-induced receptor dimerization is also strongly supported by the finding that functionally inactive receptor α chain intracellular domain mutants have a dominant negative effect when overexpressed in homologous cells (Dighe et al., 1993). Cell lines in which non-functional receptors were expressed at levels 100-fold higher than the endogenous receptors no longer responded to murine IFNγ when analyzed in a variety of assays

(IRF-1 induction, MHC class I enhancement, nitric oxide induction, and development of anti-viral activity). Thus, ligand-induced dimerization of the IFNγ receptor α chain and in particular the formation of a dimerized form of the receptor's intracellular domain is a critical first step in IFNγ receptor-mediated signal transduction.

Recent work has shown that formation of the IFNγ-IFNγ receptor α chain complex leads to recruitment of the IFNγ receptor β chain (Marsters et al., 1995). Using murine cells which expressed high numbers of both human IFNγ receptor α and β chains, an IFNγ-induced complex on cell surfaces was stabilized by chemical cross-linking and found to contain one IFNγ homodimer, two receptor α chains, and one or two receptor β chains (Marsters et al., 1995). Recently, a complex containing IFNγ and the receptor α and β chains from IFNγ treated cells was coprecipitated in the absence of chemical crosslinking (Bach et al., 1996). This result unequivocally demonstrates that the receptor α and β subunits are not preassociated on the surface of unstimulated cells but are rather induced to associate by ligand.

B. A Proposed Model of IFNγ Signal Transduction

Recent work from many laboratories has contributed much to our understanding of the mechanism of IFNγ signal transduction. This information can now be put together to form one of the most complete models of cytokine receptor signaling to date. A proposed model of IFNγ signal transduction is summarized in Figure 3. In unstimulated cells, the IFNγ receptor α and β subunits are not preassociated with each other but rather associate through their intracellular domains with inactive forms of specific Janus family kinases. JAK-1 constitutively associates with the receptor α chain and JAK-2 constitution associates with the receptor β chain. Addition of IFNγ, a homodimeric ligand, to the cells induces the rapid dimerization of receptor α chains thereby forming a site which is recognized, in a species-specific manner, by the extracellular domain of the receptor β subunit. The ligand-induced association of two β subunits with two receptor α subunits brings into close juxtaposition the intracellular domains of these proteins together with the inactive JAK enzymes that they carry. In this complex, we envision that JAK-1 and JAK-2 transactivate one another and then phosphorylate the functionally critical Y440 residue on the receptor α subunit thereby forming a paired set of Stat1 docking sites on the ligated receptor. Two Stat1 molecules then associate with the paired docking sites, are brought into close

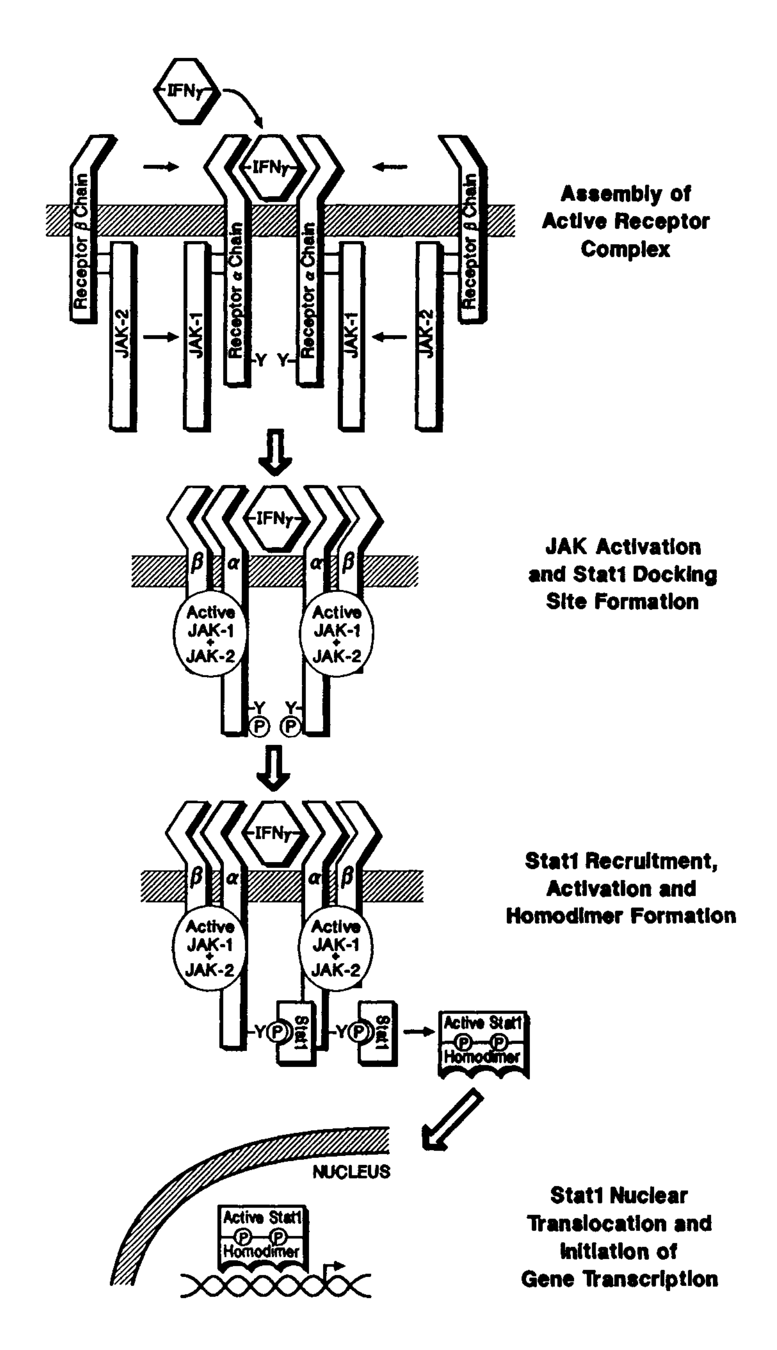


Figure 3. Proposed signaling mechanism of the IFNγ receptor.

proximity with receptor associated activated JAK enzymes, and are activated by phosphorylation of the Stat1 Y701 residue. Tyrosine phosphorylated Stat1 molecules dissociate from the receptor, form homodimeric complexes, and dissociate from their receptor tether. This activated Stat1 complex is then phosphorylated on a specific C-terminal serine residue (S723; Wen et al., 1995). Recent reports suggest that the serine phosphorylation is mediated by an as yet undefined MAP-Kinase-like enzyme (Wen et al., 1995; David et al., 1995). Activated Stat1 translocates to the nucleus and, after binding to a specific sequence in the promoter region of immediate early IFNγ inducible genes, effects gene transcription. Thus, IFNγ signaling is an ordered, affinity-driven process that derives at least some of its specificity from the specific binding of a particular Stat protein to a defined, ligand induced docking site on the activated receptor. This model of IFNγ signaling may well serve as a paradigm for a variety of other cytokine receptors that utilize the JAK-STAT pathway.

VI. IFNγ BIOLOGY

Work performed in many laboratories during the past 12 years has unequivocally established that IFNγ is an extremely pleiotropic cytokine that has unarguable physiologic importance in regulating immune and inflammatory processes. To a large extent, this research was made possible by the large-scale availability of highly purified recombinant human and murine IFNγ and the generation of neutralizing IFNγ-specific monoclonal antibodies. Most recently, three new and exciting models of genetic IFNγ deficiency in mice have been derived by ablating either IFNγ- or IFNγ receptor-gene expression using homologous recombination and embryonic stem cell technologies (Huang et al., 1993; Dalton et al., 1992) or by introducing dominant negative forms of the IFNγ receptor α chain into specific tissues using transgenic mouse technologies thereby generating mice which display tissue-specific IFNγ insensitivity (Dighe et al., 1995). It is expected that these mice will provide new and exciting insights into IFNγ biology.

There is currently a vast amount of information available concerning IFNγ's biologic activities. Herein we focus on some of the major biologic activities of this cytokine and describe them in the context of host defense and inflammatory reactions.

VII. IFNγ'S ROLE IN HOST DEFENSE

A. IFNγ as a Regulator of Response Induction

Clearly one of the major physiologic roles of IFNγ is its ability to regulate MHC class I and class II protein expression on a variety of immunologically important cell types. These include mononuclear phagocytes, endothelial cells, and epithelial cells, to name a few (Vilcek et al., 1985; Trinchieri and Perussia, 1985; Basham and Merigan, 1983; Basham et al., 1985). Interestingly, although IFNγ acts to increase class I and class II expression on most cells, it inhibits class II expression on B cells (Mond et al., 1986). Whereas IFNα and IFNβ can also up-regulate class I expression on cells, they are not inducers of MHC class II proteins. At the molecular level, IFNγ has been shown to exert its activity by regulating MHC gene transcription. MHC class I and class II genes contain *cis*-acting elements in their promoter regions that bind to IFNγ-induced *trans*-acting factors. The molecular nature of these elements and factors are currently being elucidated (Benoist and Mathis, 1990; Glimcher and Kara, 1992). At the functional level, IFNγ dependent up-regulation of MHC gene expression is an important step in promoting antigen presentation during the inductive phase of immune responses (Boss and Strominger, 1986; Grau et al., 1989; Wietzerbin et al., 1986; Stone-Wolff et al., 1984; Farber, 1990).

The monocyte/macrophage is a prime cellular target of IFNγ under physiologic conditions. Work from several laboratories has indicated that IFNγ is one of the major cytokines responsible for activating or otherwise regulating the differentiation and function of mononuclear phagocytes (Schreiber and Celada, 1985; Adams and Hamilton, 1984). IFNγ has been shown to effect the differentiation of immature myeloid precursors into mature monocytes. It promotes antigen presenting activity in macrophages, not only through the induction of MHC class II expression, but also by increasing levels of several intracellular enzymes that may be important for antigen processing (Johnson and Panitch, 1988; Allen and Unanue, 1987). In addition, it augments expression of macrophage cell surface proteins such as ICAM-1 that enhance the functional consequences of the interaction between macrophages and T cells during antigen presentation (Pober et al., 1986; Mantovani and Dejana, 1989; Frohman et al., 1989).

IFNγ also exerts its effects on other cells of the immune system. It regulates immunoglobulin isotype switching in B cells (Snapper and

Paul, 1987) and antagonizes the ability of IL-4 to induce MHC class II expression on murine B cells (Finkelman et al., 1990). These responses result from the direct effect of IFNγ on the B cell. B cell responses are also influenced indirectly by IFNγ's ability to regulate the development of specific subsets of $CD4^+$ T cells.

B. IFNγ Regulation of Th Cell Development

It is now recognized that both human and murine $CD4^+$ T cells can be divided into two largely exclusive subsets (Mosmann and Coffman, 1989). Th1 cells promote cell mediated immunity and delayed type hypersensitivity (DTH) responses by their production of IFNγ, lymphotoxin (LT), and IL-2. In contrast, Th2 cells produce IL-4, 5, 6, 10, and 13, and predominantly provide help for antibody production and regulate isotype switching. The *in vitro* and *in vivo* requirement for IFNγ in Th1 development has been long recognized (Hsieh et al., 1993a, 1993b). IFNγ appears necessary for Th1 development, but alone is not sufficient for induction of a Th1 phenotype. The ability to regulate $CD4^+$ T cell activation/differentiation thereby establishes IFNγ as a key component in determining the type of immune effector function that eventually develops during the course of an immune response. The opposing effects of IL-10 and IFNγ serve to crossregulate the development of specific immune responses. IL-10 inhibits IFNγ production by T cells and NK cells and thereby diverts the response to the humoral pole. Additionally, IFNγ production by Th1 cells may regulate the development of a Th2 response through inhibiting Th2 proliferation (Gajewski and Fitch, 1988). IFNγ has a profound antiproliferative effect on the Th2 subset of murine $CD4^+$ T cells but not on Th1 (Mosmann and Coffman, 1991; Gajewski et al., 1989b).

The role of IFNγ in *in vivo* regulation of Th phenotype has been examined in studies of murine experimental Leishmaniasis (Scott, 1991). Neutralization of IFNγ leads to an exacerbating Th2 response in resistant mice, a phenomenon also demonstrated in mice with targeted disruption of IFNγ (Wang et al., 1994). In contrast to the exacerbating effects of neutralizing IFNγ, administration of IFNγ *in vivo* during murine Leishmaniasis fails to produce a curative response in susceptible BALB/c mice (Scott, 1991). Therefore, both *in vivo* and *in vitro* data suggest that IFNγ is necessary but not sufficient for the development of the Th1 subset.

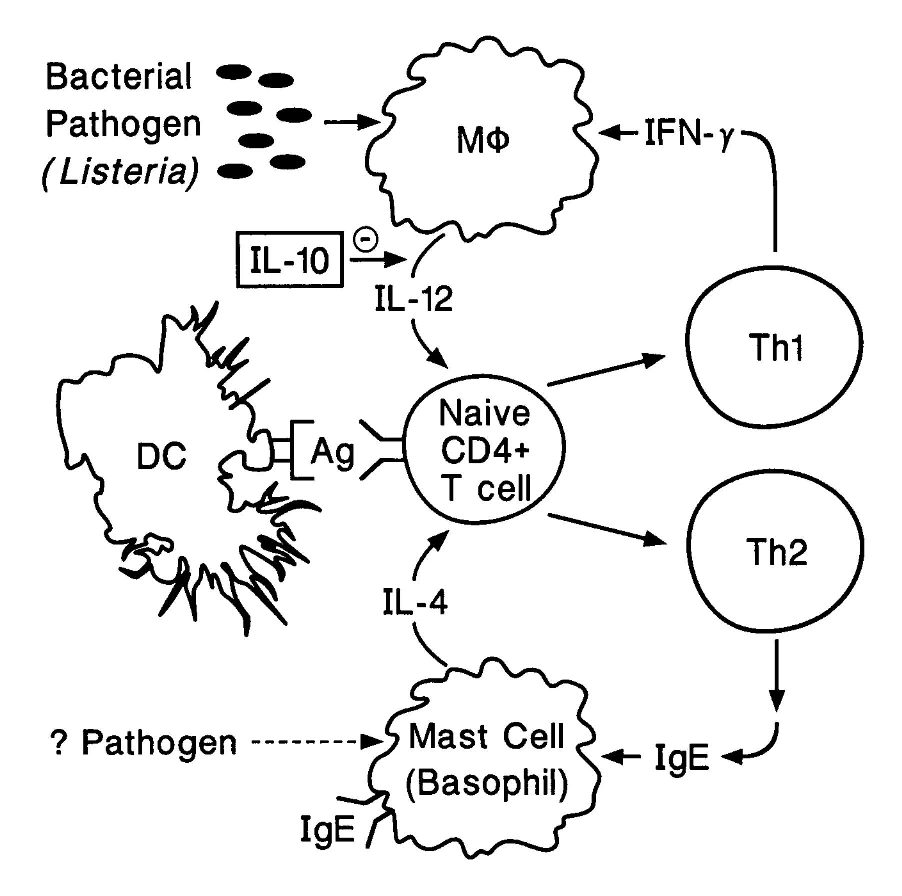


Figure 4. IFNγ's role in Th cell development. IFNγ's requirement for Th1 development is due to IFNγ's potentiation of antigen presenting cell IL-12 production. IFNγ may also regulate Th cell development by its selective anti-proliferative effect on Th2 cells

An important site of IFNγ action in Th1 development appears to be the macrophage. Transgenic mice that express dominant negative IFNγ receptor α chains in the macrophage compartment demonstrate macrophage specific unresponsiveness to IFNγ (Dighe et al., 1995). Mice with IFNγ unresponsive macrophages succumb to sublethal doses of Listeria monocytogenes and *in vitro* IFNγ unresponsive macrophages are unable to support Listeria-induced Th1 development (Dighe et al., 1995). The actions of pathogens such as Listeria for promoting Th1 development are macrophage-dependent, and rely on the production of IL-12 (Hsieh et al., 1993a, 1993b). IFNγ enhances the capacity of macrophages to

produce IL-12 in response to LPS or heat-killed Listeria. Thus, one role for IFNγ in promoting Th1 development is the potentiation of macrophage IL-12 production (Flesch et al., 1995; Dighe et al., 1995; Figure 4).

C. IFNγ as a Regulator of Effector Mechanisms

There can be little doubt that IFNγ is the major physiologic macrophage activating factor (MAF) and, therefore, is the primary cytokine responsible for inducing nonspecific cell mediated mechanisms of host defense. Work from several laboratories has unequivocally established IFNγ's ability to activate nonspecific cytocidal activity in macrophages toward a variety of intracellular and extracellular parasites and neoplastic cells (Schreiber et al., 1985; Bancroft et al., 1987; Liew et al., 1990). IFNγ induces the expression of, as yet undefined, structures on macrophages that recognize target cells and promotes the elaboration of macrophage-derived cytocidal compounds such as reactive oxygen- and reactive nitrogen-intermediates and TNFα (Ding et al., 1988). It has also been shown to reduce the susceptibility of macrophage populations to microbial infection (Bancroft et al., 1989; Schreiber, 1986). The importance of IFNγ in the clearance of microbial pathogens has been amply demonstrated using animal models. Mice pretreated with neutralizing monoclonal antibodies to IFNγ lose their capacity to resolve infection initiated with a sublethal dose of a variety of microbial pathogens such as *Listeria monocytogenes* (Bancroft et al., 1987, 1989; Buchmeier and Schreiber, 1985), *Toxoplasma gondii* (Suzuki et al., 1988), or *Leishmania major* (Green et al., 1990). The macrophage is a critical cell in elaborating a curative response to microbial infection as shown by the inability of transgenic mice with macrophage-targeted IFNγ unresponsiveness to resolve infection with *Listeria monocytogenes* (Dighe et al., 1995). Thus, these experiments document the capacity of endogenously produced IFNg to activate macrophages under physiologic *in vivo* conditions.

Currently, a great deal of attention is being focused on IFNγ's ability to induce nitric oxide (NO) production in cells. NO is a cellular product that plays an important role in effecting intracellular killing of microbial pathogens in the mouse. Nitric oxide is generated as a result of the enzymatic conversion of L-arginine to L-citrulline (Davies et al., 1984). This reaction is catalyzed by a family of enzymes known as nitric oxide synthase (NOS). At least three forms of the enzyme have been identified (Lowenstein and Snyder, 1992). Two are

expressed constitutively in a tissue-specific manner (endothelium and nervous tissue) and produce low levels of NO that function to effect cell-cell communication. In contrast, the third is an inducible form of the enzyme whose expression is controlled by two stimuli: IFN and a second signal. The signals that trigger NO production in IFNγ primed cells are a diverse group of endogenous and exogenous substances such as TNFα, IL-1, LPS, and whole bacteria (Hibbs et al., 1990; Corbett et al., 1991).

IFNγ-dependent formation of nitric oxide appears to be a major mechanism in the mouse for the macrophage-mediated killing of intracellular pathogens. Much of this information has been gleaned from the study of murine models of infection with *Leishmania* or *Listeria*. Macrophages exposed *in vitro* to IFNγ and infected with either *Leishmania* amastigotes or *Listeria* develop the capacity to kill the intracellular pathogens. Killing is completely inhibited when the macrophages are treated with competitive inhibitors of iNOS such as the L-arginine analogs, N-monomethyl-L-arginine (L-NMMA; Green et al., 1990) or aminoguanidine (Schreiber and Sheehan, 1991). *In vivo*, the important role of IFNγ-dependent induction of NO in murine models of microbial immunity has been indicated by several types of experiments. Mice undergoing active infection produce NO as detected by the presence in the urine of the stable (NO) oxidation product nitrite (NO_2). NO_2 production is blocked when mice are treated with neutralizing monoclonal antibodies specific for either IFNγ or TNF (Green et al., 1990). Additionally, mice treated with aminoguanidine succumb to infection with a sublethal dose of *Listeria monocytogenes*, much like mice treated with anti-IFNγ (Beckerman et al., 1993). Most recently, the inducible NO synthase gene has been ablated in mice using homologous recombination techniques. Importantly, these mice are unable to mount a curative response to sublethal *Listeria* infection.

In addition to enhancing *nonspecific* cell mediated cytocidal activities, IFNγ also enhances the ability of the macrophage to participate in other immune response effector functions. It increases expression of high affinity Fc receptors on monocytes/macrophages (FcγRI) and thereby enhances the capacity of these cells to participate in antibody-dependent cellular cytotoxicity (ADCC) reactions (Erbe et al., 1990). IFNγ also enhances the biosynthesis of a variety of complement proteins (such as C2, C3, C4, and Factor B) by macrophages and fibroblasts (Strunk et al., 1985) and regulates the expression of complement receptors on the mononuclear phagocyte plasma membrane thereby promoting humoral immunity through enhancement of complement activity.

VIII. IFNγ IN THE INFLAMMATORY RESPONSE

IFNγ plays an important role in promoting inflammatory reactions. It enhances production of TNFα by six to eightfold (Beutler and Cerami, 1986; Beutler et al., 1986) and induces increased expression of both the p55 and p75 TNF receptors on cell surfaces (Pfizenmaier et al., 1992). During an inflammatory response cells leave the circulation and migrate to the point of infection. During this process they must first bind to and then extravasate through the vascular endothelium. IFNγ and TNFα can promote the expression of overlapping sets of cell-surface molecules that play an important role in this process (Pober et al., 1986). For example, IFNγ by itself induces increased ICAM-1 expression in human endothelial cells and together with TNFα effects large increases in ELAM-1 expression in these cells. Thus, the ability of IFNγ and TNFα to enhance expression of cell surface adhesion molecules may serve to expand and amplify the overall inflammatory response. The cooperative ability of IFNγ and TNFα to modulate cell migration was confirmed in *in vivo* experiments (Munro et al., 1989). Skin biopsies, taken from baboons treated intracutaneously with both IFNγ and TNFα were found to contain twice the numbers of monocytes compared to animals injected with either cytokine alone. Expression of ELAM-1, ICAM-1, and MHC class I molecules was also synergistically enhanced in animals treated with both cytokines.

TNFα has long been known to mediate many of the toxic effects of LPS (Old, 1985, 1988; Beutler and Cerami, 1986) and is a key mediator in the Shwartzman reaction (a model of LPS-mediated tissue damage); Old, 1985; Movat et al., 1987). In the classical Shwartzman reaction, animals are initially treated with a local, sensitizing dose of LPS (5 μg), followed 24 hours later by a provocative intravenous dose of LPS (100 μg). The physiologic responses evoked by this protocol mimic those seen clinically in septic shock and disseminated intravascular coagulation and include hemorrhagic necrosis, fibrin and platelet mediated vascular occlusion, and accumulation of neutrophils at the local site (Old, 1988; Beutler and Cerami, 1986; Tracey et al., 1986). Using the Shwartzman reaction as a model to investigate the mechanism(s) involved in LPS-induced disease, IFNγ has been shown to play a crucial role in the progression of this inflammatory response (Billiau, 1987, 1988; Heremans et al., 1987, 1990; Billiau et al., 1987; Matsumura and Nakano, 1988; Lorence et al., 1990). Treatment of animals with IFNγ prior to sensitization with LPS leads to enhanced production of TNFα and

increased mortality (Heremans et al., 1987, 1990; Heinzel, 1990). Conversely, treatment of animals with neutralizing IFNγ-specific monoclonal antibodies prior to injection of the sensitizing dose of LPS, protects them from the pathologic effects of the provocative dose (Billiau et al., 1987). The endogenous IFNγ produced in this situation may come from NK cells which produce IFNγ in response to IL-12 and TNF produced by LPS-stimulated macrophages (Flesch et al., 1995; Dighe et al., 1995). Thus, IFNγ appears to be a key mediator in the development of the immunopathologic consequences of inflammatory responses.

IX. IFNγ IN TUMOR REJECTION

Renewed interest in the action of IFNγ in tumor biology has been prompted by the recent demonstration of the anti-tumor action of the cytokine IL-12 (Brunda et al., 1993; Nastala et al., 1994). A major activity of IL-12 is the stimulation of IFNγ production and IL-12's anti-tumor activity has been demonstrated to be IFNγ-dependent. Whereas IL-12 can induce tumor elimination in mice treated with control antibody, IL-12 displays no therapeutic activity in anti-IFNγ treated mice (Nastala et al., 1994).

There are several potential targets for IFNγ action in the process of tumor rejection. IFNγ could be important at the level of the mononuclear phagocyte to improve the processing and presentation of tumor antigens. Indeed, recent studies have demonstrated the ability of host macrophages to process and present tumor antigens (Huang et al., 1995). IFNγ also potentiates production of the cytokines TNF and IL-12 and this may serve to amplify the role of monocyte/macrophages in the rejection process. However, an unexpected site of action of IFNγ has recently been shown to be the tumor cell itself. Tumor cells rendered insensitive to IFNγ by expression of a dominant negative IFNγ receptor α chain demonstrate enhanced tumorigenicity and growth rates in syngeneic mice (Dighe et al., 1994). IFNγ's actions in this process involve alterations in the tumor cell leading to increased immunogenicity. IFNγ may act on the tumor cell to up-regulate processing and presentation of tumor antigens to $CD4^+$ and $CD8^+$ T cells. These observations suggest that responsiveness of the tumor to IFNγ may be a key determinant in the development of effective anti-tumor responses. The potent IFNγ-dependent anti-tumor actions of IL-12 suggest that IFNγ responses of both

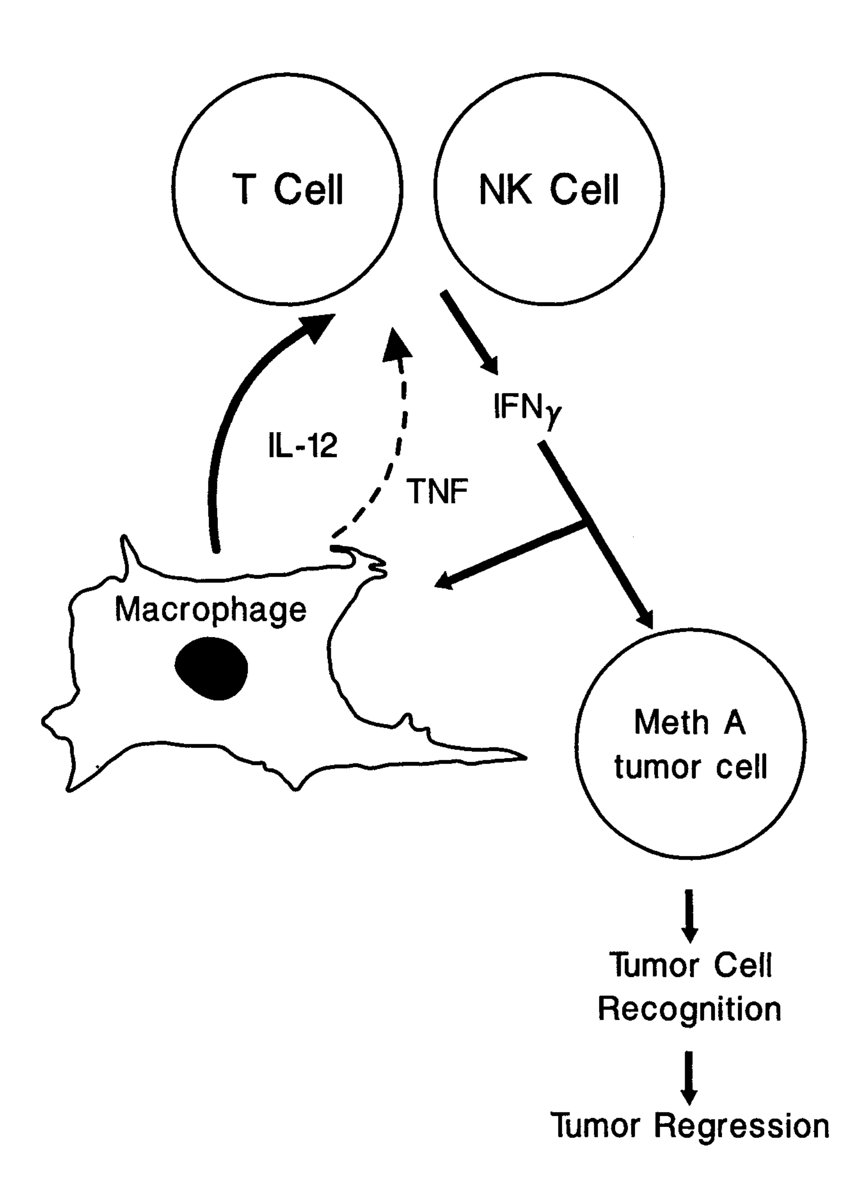


Figure 5. Model for IFNγ action in tumor rejection. IFNγ potentiates host macrophage IL-12 and TNF production, two cytokines known to be involved in tumor rejection. Additionally, IFNγ acting at the level of the tumor cell enhances tumor cell recognition and elimination by specific immunity.

host and tumor are important in the process of tumor rejection. A proposed model of IFNγ's actions in tumor rejection is presented in Figure 5.

X. CONCLUSION

The rapid advances that have been made recently in defining IFNγ's actions at the molecular level have led to a new appreciation of the physiologic roles of this cytokine *in vivo*. It is likely that this enhanced understanding will promote the development of novel procedures to regulate host responses by regulating IFNγ's actions. The next few years will be an exciting time in which basic biologists and clinicians will work together to harness the insights made at the laboratory bench into novel therapeutic strategies.

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REFERENCES

Adams, D.O., & Hamilton, T.A. (1984). The cell biology of macrophage activation. Annu. Rev. Immunol. 2, 283-318.

Aguet, M., & Merlin, G. (1987). Purification of human gamma interferon receptors by sequential affinity chromatography on immobilized monoclonal anti-receptor antibodies and human gamma interferon. J. Exp. Med. 165, 988-999.

Aguet, M., Dembic, Z., & Merlin, G. (1988). Molecular cloning and expression of the human interferon-γ receptor. Cell 55, 273-280.

Allen, P.M., & Unanue, E.R. (1987). Antigen processing and presentation at a molecular level. Adv. Exp. Med. Biol. 225, 147-154.

Anderson, P., Yip, Y.K., & Vilĉek, J. (1982). Specific binding of ^{125}I-human interferon-gamma to high affinity receptors on human fibroblasts. J. Biol. Chem. 257, 11301-11304.

Arakawa, T., Hsu, Y.R., Parker, C.G., & Lai, P.H. (1986). Role of polycationic C-terminal portion in the structure and activity of recombinant human interferon-gamma. J. Biol. Chem. 261, 8534-8539.

Bach, E.A., Tanner, J.W., Marsters, S.A., Ashkenazi, A., Aguet, M., Shaw, A.S., & Schreiber, R.D. (1996). Ligand induced assembly and activation of the gamma interferon receptor in intact cells. Mol. Cell. Biol. 16, 3214-3221.

Bancroft, G.J., Schreiber, R.D., Bosma, G.C., Bosma, M.J., & Unanue, E.R. (1987). A T cell-independent mechanism of macrophage activation by interferon-gamma. J. Immunol. 139, 1104-1107.

Bancroft, G.J., Sheehan, K.C.F., Schreiber, R.D., & Unanue, E.R. (1989). Tumor necrosis factor is involved in the T cell-independent pathway of macrophage activation in scid mice. J. Immunol. 143, 127-130.

Bancroft, G.J., Schreiber, R.D., & Unanue, E.R. (1991). Natural immunity: A T-cell-independent pathway of macrophage activation, defined in the SCID mouse. Immunol. Rev. 124, 5-24.

Basham, T.Y., & Merigan, T.C. (1983). Recombinant interferon-gamma increases HLA-DR synthesis and expression. J. Immunol. 130, 1492-1494.

Basham, T.Y., Nickoloff, B.J., Merigan, T.C., & Morhenn, V.B. (1985). Recombinant gamma interferon differentially regulates class II antigen expression and biosynthesis on cultured normal human keratinocytes. J. Interferon Res. 5, 23-32.

Basu, M., Pace, J.L., Pinson, D.M., Hayes, M.P., Trotta, P.P., & Russell, S.W. (1988). Purification and partial characterization of a receptor protein for mouse interferon gamma. Proc. Natl. Acad. Sci. USA 85, 6282-6286.

Bazan, J.F. (1990). Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA 87, 6934-6938.

Beckerman, K.P., Rogers, H.W., Corbett, J.A., Schreiber, R.D., McDaniel, M.L., & Unanue, E.R. (1993). Release of nitric oxide during the T cell-independent pathway of macrophage activation. Its role in resistance to *Listeria* monocytogenes. J. Immunol. 150, 888-895.

Benoist, C., & Mathis, D. (1990). Regulation of major histocompatibility complex class II genes: X, Y, and other letters of the alphabet. Annu. Rev. Immunol. 8, 681-716.

Beutler, B., & Cerami, A. (1986). Cachectin and tumour necrosis factor as two sides of the same biological coin. Nature 320, 584-588.

Beutler, B., Tkacenko, V., Milsark, I., Krochin, N., & Cerami, A. (1986). Effect of gamma interferon on cachectin expression by mononuclear phagocytes. Reversal of the lps-d (endotoxin resistance) phenotype. J. Exp. Med. 164, 1791-1796.

Billiau, A. (1987). Interferons and inflammation. J. Interferon Res. 7, 559-567.

Billiau, A. (1988). Gamma-interferon: The match that lights the fire? Immunol. Today 9, 37-40.

Billiau, A., Heremans, H., Vandekerckhove, F., & Dillen, C. (1987). Anti-interferon gamma antibody protects mice against the generalized Shwartzman reaction. Eur. J. Immunol. 17, 1851.

Boss, J.M., & Strominger, J.L. (1986). Regulation of a transfected human class II major histocompatibility complex gene in human fibroblasts. Proc. Natl. Acad. Sci. USA 83, 9139-9143.

Brunda, M.J., Luistro, L., Warrier, R.R., Wright, R.B., Hubbard, B.R., Murphy, M., Wolf, S.F., & Gately, M.K. (1993). Antitumor and antimetastatic activity of interleukin 12 against murine tumors. J. Exp. Med. 178, 1223-1230.

Buchmeier, N.A., & Schreiber, R.D. (1985). Requirement of endogenous interferon-gamma production for resolution of Listeria monocytogenes infection. Proc. Natl. Acad. Sci. USA 82, 7404-7408.

Calderon, J., Sheehan, K.C.F., Chance, C., Thomas, M.L., & Schreiber, R.D. (1988). Purification and characterization of the human interferon-gamma receptor from placenta. Proc. Natl. Acad. Sci. USA 85, 4837-4841.

Chan, S.H., Kobayashi, M., Santoli, D., Perussia, B., & Trinchieri, G. (1992). Mechanisms of IFN-gamma induction by natural killer cell stimulatory factor (NKSF/IL-12): Role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. J. Immunol. 148, 92-98.

Chang, T.W., McKinney, S., Liu, V., Kung, P.C., Vilcek, J., & Le, J. (1984). Use of monoclonal antibodies as sensitive and specific probes for biologically active human gamma-interferon. Proc. Natl. Acad. Sci. USA 81, 5219-5222.

Cofano, F., Moore, S.K., Tanaka, S., Yuhki, N., Landolfo, S., & Applella, E. (1990). Affinity purification, peptide analysis, and cDNA sequence of the mouse interferon-γ receptor. J. Biol. Chem. 265, 4064-4071.

Cook, J.R., Jung, V., Schwartz, B., Wang, P., & Pestka, S. (1992). Structural analysis of the human interferon-gamma receptor: A small segment of the intracellular domain is specifically required for class I major histocompatibility complex antigen induction and antiviral activity. Proc. Natl. Acad. Sci. USA 89, 11317-11321.

Corbett, J.A., Lancaster, J.R., Jr., Sweetland, M.A., & McDaniel, M.L. (1991). Interleukin-1β-induced formation of EPR-detectable iron-nitrosyl complexes in Islets of Langerhans. J. Biol. Chem. 266, 21351.

Dalton, D.K., Pitts-meek, S., Figari, I.S.F., Keshar, S., Bradley, A., & Stewart, T.A. (1992). Mice with a targeted mutation of the IFNγ gene: Multiple defects in immune cell function. J. Interferon Res. (Abstr.)

Darnell, J.E.,Jr., Kerr, I.M., & Stark, G.R. (1994). JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

David, M., Petricoin , E., III, Bejamin, C., Pine, R., Weber, M.J., & Larner, A.C. (1995). Requirement of MAP kinase (ERK2) activity in interferon α- and interferon β-stimulated gene expression through STAT proteins. Science 269, 1721-1723.

Davies, P., Bailey, P.J., & Goldenberg, M.M. (1984). The role of arachidonic acid oxygenation products in pain and inflammation. Ann. Rev. Immunol. 2, 335-357.

De Maeyer, E. (1984). The interferon system and the immune system. In: *Interferons and the Immune System* (Vilcek, J., & De Maeyer, E., Eds.). Elsevier, Amsterdam, pp. 1-6.

Derynck, R., Leung, D.W., Gray, P.W., & Goeddel, D.V. (1982). Human interferon gamma is encoded by a single class of mRNA. Nucleic Acids Res. 10, 3605-3615.

Dighe, A.S., Farrar, M.A., & Schreiber, R.D. (1993). Inhibition of cellular responsiveness to interferon-γ (IFNγ) induced by overexpression of inactive forms of the IFNγ receptor. J. Biol. Chem. 268, 10645-10653.

Dighe, A.S., Richards, E., Old, L.J., & Schreiber, R.D. (1994). Enhanced *in vivo* growth and resistance to rejection of tumor cells expressing dominant negative IFNγ receptors. Immunity 1, 447-456.

Dighe, A.S., Campbell, D., Hsieh, C-S., Clarke, S., Greaves, D.R., Gordon, S., Murphy, K.M., & Schreiber, R.D. (1995). Tissue specific targeting of cytokine unresponsiveness in transgenic mice. Immunity 3, 657-666.

Ding, A.H., Nathan, C.F., & Stuehr, D.J. (1988). Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages: Comparison of activating cytokines and evidence for independent production. J. Immunol. 141, 2407-2412.

Ealick, S.E, Cook, W.J., Vijay-Kumar, S., Carson, M., Nagabhushan, T.L., Trotta, P.P., & Bugg, C.E. (1991a). Three-dimensional structure of recombinant human interferon-gamma. Science 252, 698-702.

Erbe, D.V., Collins, J.E., Shen, L., Graziano, R.F., & Fanger, M.W. (1990). The effect of cytokines on the expression and function of Fc receptors for IgG on human myeloid cells. Mol. Cell. Biol. 27, 57-67.

Farber, J.M. (1990). A macrophage mRNA selectively induced by gamma-interferon encodes a member of the platelet factor 4 family of cytokines. Proc. Natl. Acad. Sci. USA 87, 5238-5242.

Farrar, M.A., & Schreiber, R.D. (1993). The molecular cell biology of interferon-γ and its receptor. Annu. Rev. Immunol. 11, 571-611.

Farrar, M.A., Fernandez-Luna, J., & Schreiber, R.D. (1991). Identification of two regions within the cytoplasmic domain of the human interferon-gamma receptor required for function. J. Biol. Chem. 266, 19626-19635.

Farrar, M.A., Campbell, J.D., & Schreiber, R.D. (1992a). A point mutational analysis of the intracellular domain of human IFNg receptor. J. Interferon Res.

Farrar, M.A., Campbell, J.D. & Schreiber, R.D. (1992b). Identification of a functionally important sequence motif in the carboxy terminus of the interferon-g receptor. Proc. Natl. Acad. Sci. USA 89, 11706-11710.

Finbloom, D.S., Hoover, D.L., & Wahl, L.M. (1985). The characteristics of binding of human recombinant interferon-gamma to its receptor on human monocytes and human monocyte-like cell lines. J. Immunol. 135, 300-305.

Finkelman, F.D., Holmes, J., Katona, I.M., Urban, J.F., Jr., Beckmann, M.P., Park, L.S., Schooley, K.A., Coffman, R.L., Mosmann, T.R., & Paul, W.E. (1990). Lymphokine control of *in vivo* immunoglobulin isotype selection. Annu. Rev. Immunol. 8, 303-333.

Fischer, T., Rehm, A., Aguet, M., & Pfizenmaier, K. (1990a). Human chromosome 21 is necessary and sufficient to confer human IFNγ responsiveness to somatic cell hybrids expressing the cloned human IFNγ receptor gene. Cytokine 2, 157-161.

Fischer, T., Thoma, B., Scheurich, P., & Pfizenmaier, K. (1990b). Glycosylation of the human interferon-gamma receptor. N-linked carbohydrates contribute to structural heterogeneity and are required for ligand binding. J. Biol. Chem. 265, 1710-1717.

Flesch, I., Hess, J.H., Huang, S., Aguet, M., Rothe, J., Bluethmann, H., & Kaufmann, S.H.E. (1995). Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon γ and tumor necrosis factor α. J. Exp. Med. 181, 1615-1621.

Fountoulakis, M., Kania, M., Ozmen, L., Loetscher, H.R., Garotta, G., & van Loon, A.P. (1989). Structure and membrane topology of the high-affinity receptor for human IFN-gamma: Requirements for binding IFN-gamma. One single 90-kilodalton IFN-gamma receptor can lead to multiple cross-linked products and isolated proteins. J. Immunol. 143, 3266-3276.

Fountoulakis, M., Zulauf, M., Lustig, A., & Garotta, G. (1992). Stoichiometry of interaction between interferon-γ and its receptor. Eur. J. Biochem. 208, 781-787.

Frohman, E.M., Frohman, T.C., Dustin, M.L., Vayuvegula, B., Choi, B., Gupta, A., van den Noort, S., & Gupta, S. (1989). The induction of intercellular adhesion molecule 1 (ICAM-1) expression on human fetal astrocytes by interferon-gamma,

tumor necrosis factor alpha, lymphotoxin, and interleukin-1: Relevance to intracerebral antigen presentation. J. Neuroimmunol. 23, 117-124.

Gajewski, T.F., & Fitch, F.W. (1988). Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. J. Immunol. 140, 4245-4252.

Gajewski, T.F., Joyce, J., & Fitch, F.W. (1989a). Antiproliferative effect of IFN-gamma in immune regulation. III. Differential selection of TH1 and TH2 murine helper T lymphocyte clones using recombinant IL-2 and recombinant IFN-gamma. J. Immunol. 143, 15-22.

Gajewski, T.F., Schell, S.R., Nau, G., & Fitch, F.W. (1989b). Regulation of T-Cell Activation: Differences among T cell subsets. Immunol. Rev. 111, 79-110.

Glimcher, L.H., & Kara, C.J. (1992). Sequences and factors: A guide to MHC class II transcription. Annu. Rev. Immunol. 10, 13-50.

Grau, G.E., Heremans, H., Piguet, P.F., Pointaire, P., Lambert, P.H., Billiau, A., & Vassalli, P. (1989). Monoclonal antibody against interferon gamma can prevent experimental cerebral malaria and its associated overproduction of tumor necrosis factor. Proc. Natl. Acad. Sci. USA 86, 5572-5574.

Gray, P.W., & Goeddel, D.V. (1982). Structure of the human immune interferon gene. Nature 298, 859-863.

Gray, P.W., & Goeddel, D.V. (1983). Cloning and expression of murine immune interferon cDNA. Proc. Natl. Acad. Sci. USA 80, 5842-5846.

Gray, P.W., Leung, D.W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, C.C., Derynck, R., Sherwood, P.J., Wallace, D.M., Berger, S.L., Levinson, A.D., & Goeddel, D.V. (1982). Expression of human immune interferon cDNA in *E. coli* and monkey cells. Nature 295, 503-508.

Gray, P.W., Leong, S., Fennie, E.H., Farrar, M.A., Pingel, J.T., Fernandez-Luna, J., & Schreiber, R.D. (1989). Cloning and expression of the cDNA for the murine interferon gamma receptor. Proc. Natl. Acad. Sci. USA 86, 8497-8501.

Green, S.J., Crawford, R.M., Hockmeyer, J.T., Meltzer, M.S., & Nacy, C.A. (1990). Leishmania major amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumor necrosis factor-alpha. J. Immunol. 145, 4290-4297.

Greenlund, A.C., Schreiber, R.D., Goeddel, D.V., & Pennica, D. (1993). Interferon-γ induces receptor dimerization in solution and on cells. J. Biol. Chem. 268, 18103-18110.

Greenlund, A.C., Farrar, M.A., Viviano, B.L., & Schreiber, R.D. (1994). Ligand induced IFNγ receptor phosphorylation couples the receptor to its signal transduction system (p91). EMBO J. 13, 1591-1600.

Greenlund, A.C., Morales, M.O., Viviano, B.L., Yan, H., Krolewski, J., & Schreiber, R.D. (1995). Stat recruitment by tyrosine-phosphorylated cytokine receptors: An ordered reversible affinity-driven process. Immunity 2, 677-687.

Handa, K., Suzuki, R., Matsui, H., Shimizu, Y., & Kumagai, K. (1983). Natural killer (NK) cells as a responder to interleukin 2 (IL 2). II. IL 2-induced interferon gamma production. J. Immunol. 130, 988-992.

Heinzel, F.P. (1990). The role of IFN-gamma in the pathology of experimental endotoxemia. J. Immunol. 145, 2920-2924.

Hemmi, S., Peghini, P., Metzler, M., Merlin, G., Dembic, Z., & Aguet, M. (1989). Cloning of murine interferon gamma receptor cDNA: Expression in human cells mediates high-affinity binding but is not sufficient to confer sensitivity to murine interferon gamma. Proc. Natl. Acad. Sci. USA 86, 9901-9905.

Hemmi, S., Bohni, R., Stark, G., DiMarco, F., & Aguet, M. (1994). A novel member of the interferon receptor family complements functionality of the murine interferon-gamma receptor in human cells. Cell 76, 803-810.

Heremans, H., Dijkmans, R., Sobis, H., Vandekerckhove, F., & Billiau, A. (1987). Regulation by interferons of the local inflammatory response to bacterial lipopolysaccharide. J. Immunol. 138, 4175-4179.

Heremans, H., Van Damme, J., Dillen, C., Dijkmans, R., & Billiau, A. (1990). Interferon gamma, a mediator of lethal lipopolysaccharide-induced Shwartzman-like shock reactions in mice. J. Exp. Med. 171, 1853-1869.

Hershey, G.K., & Schreiber, R.D. (1989). Biosynthetic analysis of the human interferon-gamma receptor. Identification of N-linked glycosylation intermediates. J. Biol. Chem. 264, 11981-11988.

Hibbs, J.B., Jr., Taintor, R.R., Vavrin, Z., Granger, D.L., Drapier, J-C., Amber, I.J., & Lancaster, J.R., Jr. (1990). Synthesis of nitric oxide from a terminal guanidino nitrogen atom of L-arginine: A molecular mechanism regulating cellular proliferation that targets intracellular iron. In: *Nitric Oxide from L-arginine: A Bioregulatory System* (Moncado, S., & Higgs, E.A., Eds.). Elsevier Science, Amsterdam, pp. 189-222.

Hibino, Y., Mariano, T.M., Kumar, C.S., Kozak, C.A., & Pestka, S. (1991). Expression and reconstitution of a biologically active mouse interferon gamma receptor in hamster cells. Chromosomal location of an accessory factor. J. Biol. Chem. 266, 6948-6951.

Ho, A.S.Y., Liu, Y., Khan, T.A., Hsu, D-H., Bazan, J.F., & Moore, K.W. (1993). A receptor for interleukin-10 is related to interferon receptors. Proc. Natl. Acad. Sci. USA 90, 11267-11271.

Hsieh, C.-S., Macatonia, S., Tripp, C.S., Wolf, S.F., O'Garra, A., & Murphy, K.M. (1993a). Development of Th1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. Science 260, 547-549.

Hsieh, C.-S., Macatonia, S.E., O'Garra, A., & Murphy, K.M. (1993b). Pathogen-induced Th1 phenotype development in $CD4^+$ αβ-TCR transgenic T cells is macrophage dependent. Intl. Immunol. 5, 371-382.

Huang, S., Hendriks, W., Althage, A., Hemmi, S., Bluethmann, H., Kamijo, R., Vilcek, J., Zinkernagel, R., & Aguet, M. (1993). Immune response in mice that lack the interferon-γ receptor. Science 259, 1742-1745.

Huang, A.Y., Golumbek, P., Ahmadzadeh, M., Jaffee, E., Pardoll, D., & Levitsky, H. (1995). Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. Science 264, 961-965.

Igarashi, K., Garotta, G., Ozmen, L., Ziemiecki, A., Wilks, A.F., Harpur, A.G., Larner, A.C., & Finbloom, D.S. (1994). Interferon-gamma induces tyrosine phosphorylation of interferon-gamma receptor and regulated association of protein tyrosine kinases, Jak1 and Jak2, with its receptor. J. Biol. Chem. 269, 14333-14336.

Johnson, K.P., & Panitch, H.S. (1988). Effects of experimental recombinant interferons on multiple sclerosis. Trans. Am. Clin. Climatolog. Assoc. 100, 171-176.

Jung, V., Rashidbaigi, A., Jones, C., Tischfield, J.A., Shows, T.B., & Pestka, S. (1987). Human chromosomes 6 and 21 are required for sensitivity to human interferon gamma. Proc. Natl. Acad. Sci. USA 84, 4151-4155.

Jung, V., Jones, C., Kumar, C.S., Stefanos, S., O'Connell, S., & Pestka, S. (1990). Expression and reconstitution of a biologically active human IFNγ receptor in hamster cells. J. Biol. Chem. 265, 1827-1830.

Kaplan, D.K., Greenlund, A.C., Tanner, J.W., Shaw, A.S., & Schreiber, R.D. (1996). Identification of an interferon-γ receptor α chain sequence required for JAK-1 binding. J. Biol. Chem. 271, 9-12.

Kotenko, S., Izotova, L., Pollack, B., Mariano, T., Donnelly, R., Muthukumaran, G., Cook, J., Garrotta, G., Silvennoinen, O., Ihle, J., & Pestka, S. (1995). Interaction between the components of the interferon gamma receptor complex. J. Biol. Chem. 270, 20915-20912.

Kumar, C.S., Muthukumaran, G., Frost, L.J., Noe, M., Ahn, Y.H., Mariano, T.M., & Pestka, S. (1989). Molecular characterization of the murine interferon gamma receptor cDNA. J. Biol. Chem. 264, 17939-17946.

Langer, J.S., & Pestka, S. (1988). Interferon receptors. Immunol. Today 9, 393-400.

Le, J., Chang, T.W., Liu, V., Yip, Y.K., & Vilcek, J. (1985). Monoclonal antibodies as structural probes for oligomeric human interferon-gamma. J. Interferon Res. 5, 445-453.

Liew, F.Y., Li, Y., & Millott, S. (1990). Tumor necrosis factor-alpha synergizes with IFN-gamma in mediating killing of *Leishmania* major through the induction of nitric oxide. J. Immunol. 145, 4306-4310.

Littman, S.J., Faltynek, C.R., & Baglioni, C. (1985). Binding of human recombinant 125I-interferon gamma to receptors on human cells. J. Biol. Chem. 260, 1191-1195.

Lorence, R.M., Edwards, C.K., Walter, R.J., Kelley, K.W., & Greager, J.A. (1990). In vivo effects of recombinant interferon-gamma: Augmentation of endotoxin-induced necrosis of tumors and priming of macrophages for tumor necrosis factor-alpha production. Cancer Lett. 53, 223-229.

Lowenstein, C.J., & Snyder, S.H. (1992). Nitric oxide, a novel biologic messenger. Cell 70, 705-707.

Mantovani, A., & Dejana, E. (1989). Cytokines as communication signals between leukocytes and endothelial cells. Immunol. Today 10, 370-375.

Mao, C., Aguet, M., & Merlin, G. (1989). Molecular characterization of the human interferon-gamma receptor: Analysis of polymorphism and glycosylation. J. Interferon Res. 9, 659-669.

Marsters, S., Pennica, D., Bach, E., Schreiber, R.D., & Ashkenazi, A. (1995). Interferon γ signals via a high-affinity multisubunit receptor complex that contains two types of polypeptide chain. Proc. Natl. Acad. Sci. USA 92, 5401-5405.

Matsumura, H., & Nakano, M. (1988). Endotoxin-induced interferon-gamma production in culture cells derived from BCG-infected C3H/HeJ mice. J. Immunol. 140, 494-500.

Miura, O., Cleveland, J.L., & Ihle, J.N. (1993). Inactivation of erythropoietin receptor function by point mutation in a region having homology with other cytokine receptors. Mol. Cell. Biol. 13, 1788-1795.

Mond, J.J., Carman, J., Sarma, C., Ohara, J., & Finkelman, F.D. (1986). Interferon-gamma suppresses B cell stimulation factor (BSF-1) induction of class II MHC determinants on B cells. J. Immunol. 137, 3534-3537.

Moore, K.W., O'Garra, A.O., de Waal Malefyt, R., Vieira, P., & Mosmann, T.R. (1993). Interleukin-10. Ann. Rev. Immunol. 11, 165-190.

Mosmann, T.R., & Coffman, R.L. (1989). TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. 7, 145-173.

Movat, H.Z., Burrowes, C.E., Cybulsky, M.I., & Dinarello, C.A. (1987). Acute inflammation and a Shwartzman-like reaction induced by interleukin-1 and tumor necrosis factor. Synergistic action of the cytokines in the induction of inflammation and microvascular injury. Am. J. Pathol. 129, 463-476.

Müller, M., Briscoe, J., Laxton, C., Guschin, D., Zlemleckl, A., Silvennoinen, O., Harpur, A.G., Barbier, G., Witthuhn, B.A., Schindler, C., Pellegrini, S., Wilks, A.F., Ihle, J.N., Stark, G.R., & Kerr, I.M. (1993). The protein tyrosine kinase JAK1 complements a mutant cell line defective in the interferon-alpha/beta and -gamma signal transduction pathways. Nature 366, 129-135.

Munro, S., & Maniatis, T. (1989). Expression and cloning of the murine interferon-γ receptor cDNA. Proc. Natl. Acad. Sci. USA 86, 9248-9252.

Munro, J.M., Pober, J.S., & Cotran, R.S. (1989). Tumor necrosis factor and interferon-gamma induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of papio anubis. Am. J. Pathol. 135, 121-131.

Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., & Kishimoto, T. (1991). Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. Proc. Natl. Acad. Sci. USA 88, 11349-11353.

Nagata, K., Izumi, T., Kitagawa, T., & Yoshida, N. (1987). Oligomeric structure of recombinant human and murine immune interferons by means of sedimentation equilibrium. J. Interferon Res. 7, 313-320.

Nastala, C.L., Edington, H.D., McKinney, T.G., Tahara, H., Nalesnik, M.A., Brunda, M.J., Gately, M.K., Wolf, S.F., Schreiber, R.D., Storkus, W.J., & Lotze, M.T. (1994). Recombinant IL-12 administration induces tumor regression in association with IFN-γ production. J. Immunol. 153, 1697-1706.

Naylor, S.L., Sakaguchi, A.Y., Shows, T.B., Law, M.L., Goeddel, D.V., & Gray, P.W. (1983). Human immune interferon gene is located on chromosome 12. J. Exp. Med. 157, 1020-1027.

Naylor, S.L., Gray, P.W., & Lalley, P.A. (1984). Mouse immune interferon (IFNγ) gene is on human chromosome 10. Somat. Cell. Mol. Genet. 10, 531-534.

Novick, D., Orchansky, P., Revel, M., & Rubinstein, M. (1987). The human interferon-gamma receptor. Purification, characterization, and preparation of antibodies. J. Biol. Chem. 262, 8483-8487.

Old, L.J. (1985). Tumor necrosis factor (TNF). Science 230, 630-632.

Old, L.J. (1988). Tumor necrosis factor. Scientific Am. 258, 59-75.

Pace, J.L., Russell, S.W., LeBlanc, P.A., & Murasko, D.M. (1985). Comparative effects of various classes of mouse interferons on macrophage activation for tumor cell killing. J. Immunol. 134, 977-981.

Pestka, S., Langer, J.A., Zoon, K.C., & Samuel, C.E. (1987). Interferons and their actions. Annu. Rev. Biochem. 56, 727-777.

Pfizenmaier, K., Wiegmann, K., Scheurich, P., Krönke, M., Merlin, G., Aguet, M., Knowles, B.B., & Ucer, U. (1988). High affinity human IFN-gamma-binding capacity is encoded by a single receptor gene located in proximity to c-ros on human chromosome region 6q16 to 6q22. J. Immunol. 141, 856-860.

Pfizenmaier, K., Himmler, A., Schutze, S., Scheurich, P., & Kronke, M. (1992). TNF receptors and TNF signal transduction. In: *Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine* (Beutler, B., Ed.). Raven Press, Ltd., New York, pp. 439-472.

Pober, J.S., Gimbrone, M.A., Jr., Lapierre, L.A., Mendrick, D.L., Fiers, W., Rothlein, R., & Springer, T.A. (1986). Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J. Immunol. 137, 1893-1896.

Sakatsume, M., Igarashi, K., Winestock, K.D., Garotta, G., Larner, A.C., & Finbloom, D.S. (1995). The Jak kinases differentially associate with the alpha and beta (Accessory Factor) chains of the interferon gamma receptor to form a functional receptor unit capable of activating STAT transcription factors. J. Biol. Chem. 270, 17528-17534.

Scahill, S.J., Devos, R., Van der Heyden, J., & Fiers, W. (1983). Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells. Proc. Natl. Acad. Sci. USA 80, 4654-4658.

Schindler, C., Shuai, K., Prezioso, V.R., & Darnell, J.E., Jr. (1992). Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 257, 809-813.

Schreiber, R.D. (1986). Validation of a role for endogenously produced IFNγ in resolution of *Listeria Monocytogenes* infection in mice. In: *Host Defenses and Immunomodulation to Intracellular Pathogens* (Eisenstein, T.K., Bullock, W.E., & Hanna, N., Eds.). Plenum Publishing Co., Philadelphia.

Schreiber, R.D. ,& Celada, A. (1985). Molecular characterization of interferon gamma as a macrophage activating factor. Lymphokines 11, 87-118.

Schreiber, R.D., & Aguet, M. (1994). The interferon-γ receptor. In: *Guidebook to Cytokines and Their Receptors.* Oxford University Press, New York, pp. 120-123.

Schreiber, R.D., Pace, J.L., Russell, S.W., Altman, A., & Katz, D.H. (1983). Macrophage-activating factor produced by a T cell hybridoma: Physiochemical and biosynthetic resemblance to gamma-interferon. J. Immunol. 131, 826-832.

Schreiber, R.D., & Sheehan, K.C.F. (1991). The use of monoclonal antibodies to study the physiologic roles of murine cytokines. In: *Cytokine Interactions and Their Control* (Baxter, A., & Ross, R., Eds.). John Wiley & Sons, New York, pp. 105-116.

Schreiber, R.D., Hicks, L.J., Celada, A., Buchmeier, N.A., & Gray, P.W. (1985). Monoclonal antibodies to murine gamma-interferon which differentially modulate macrophage activation and antiviral activity. J. Immunol. 134, 1609-1618.

Scott, P. (1991). IFN-gamma modulates the early development of Th1 and Th2 responses in a murine model of cutaneous Leishmaniasis. J. Immunol. 147, 3149-3155.

Shuai, K., Schindler, C., Prezioso, V.R., & Darnell, J.E., Jr. (1992). Activation of transcription by IFN-γ: Tyrosine phosphorylation of a 91-kD DNA binding protein. Science 258, 1808-1812.

Snapper, C.M., & Paul, W.E. (1987). Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science 236, 944-947.

Soh, J., Donnely, R.O., Kotenko, S., Mariano, T.M., Cook, J.R., Wang, N., Emanuel, S., Schwartz, B., Miki, T., & Pestka, S. (1994). Identification and sequence of an accessory factor required for activation of the human IFN-gamma receptor. Cell 76, 793-802.

Stefanos, S., Ahn, Y.H., & Pestka, S. (1989). Characterization of human interferon-gamma receptor purified from placenta. J. Interferon Res. 9, 719-730.

Stern, A.S., Podlaski, F.J., Hulmes, J.D., Pan, Y.E., Quinn, P.M., Wolitzky, A.G., Familletti, P.C., Stremlo, D.L., Truitt, T., Chizzonite, R., & Gately, M.K. (1990). Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. Proc. Natl. Acad. Sci. USA 87, 6808-6812.

Stewart, W.E. (1979). *The Interferons*. Springer-Verlag, New York.

Stone-Wolff, D.S., Yip, Y.K., Kelker, H.C., Le, J., Henriksen-DeStefano, D., Rubin, B.Y., Rinderknecht, E., Aggarwal, B.B., & Vilĉek, J. (1984). Interrelationships of human interferon-gamma with lymphotoxin and monocyte cytotoxin. J. Exp. Med. 159, 828-843.

Street, N.E., Schumacher, J.H., Fong, T.A.T., Bass, H., Fiorentino, D.F., Leverah, J.A., & Mosmann, T.R. (1990). Heterogeneity of mouse helper T cells. Evidence from bulk cultures and limiting dilution cloning for precursors of Th1 and Th2 cells. J. Immunol. 144, 1629-1639.

Strunk, R.C., Cole, F.S., Perlmutter, D.H., & Colten, H.R. (1985). gamma-Interferon increases expression of class III complement genes C2 and factor B in human monocytes and in murine fibroblasts transfected with human C2 and factor B genes. J. Biol. Chem. 260, 15280-15285.

Suzuki, Y., Orellana, M.A., Schreiber, R.D., & Remington, J.S. (1988). Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii.* Science 240, 516-518.

Tracey, K.J., Beutler, B., Lowry, S.F., Merryweather, J., Wolpe, S., Milsark, J.W., Hariri, R.J., Fahey, T.J., Zentella, A., Albert, J.P., Shures, G.T., & Cerami, A. (1986). Shock and tissue injury induced by recombinant human cachectin. Science 234, 470-474.

Trent, J.M., Olson, S., & Lawn, R.M. (1982). Chromosomal localization of human leukocyte, fibroblast, and immune interferon genes by means of *in situ* hybridization. Proc. Natl. Acad. Sci. USA 79, 7809-7813.

Trinchieri, G., & Perussia, B. (1985). Immune interferon: A pleiotropic lymphokine with multiple effects. Immunol. Today 6, 131-136.

Tripp, C.S., Wolf, S.F., & Unanue, E.R. (1993). Interleukin-12 and tumor necrosis factor alpha are costimulators of interferon-gamma production by natural killer cells in severe combined immunodeficiency mice with Listeriosis, and interleukin-10 is a physiologic antagonist. Proc. Natl. Acad. Sci. USA 90, 3725-3729.

Ucer, U., Bartsch, H., Scheurich, P., Berkovic, D., Ertel, C., & Pfizenmaier, K. (1986). Quantitation and characterization of gamma-interferon receptors on human tumor cells. Cancer Res. 46, 5339-5343.

Van Loon, A.P.G.M., Ozmen, L., Fountoulakis, M., Kania, M., Haiker, M., & Garotta, G. (1991). High-affinity receptor for interferon-gamma (IFN-gamma), a ubiquitous protein occurring in different molecular forms on human cells: Blood monocytes and eleven different cell lines have the same IFN-gamma receptor protein. J. Leuko. Biol. 49, 462-473.

Vilcek, J., Gray, P.W., Rinderknecht, E., & Sevastopoulos, C.G. (1985). Interferon gamma: a lymphokine for all seasons. Lymphokines 11, 1-32.

Walter, M.R., Windsor, W.T., Nagabhushan, T.L., Lundell, D.J., Lunn, C.A., Zauodny, P.J., & Narula, S.W. (1995). Crystal structure of a complex between interferon-γ and its soluble high-affinity receptor. Nature 376, 230-235.

Wang, Z.-E., Reiner, S.L., Zheng, S., Dalton, D.K., & Locksley, R.M. (1994). CD4+ effector cells default to the Th2 pathway in interferon-gamma deficient mice infected with Leishmania major. J. Exp. Med. 179, 1367-1371.

Watling, D., Guschin, D., Muller, M., Silvennoinen, O., Witthuhn, B.A., Quelle, F.W., Rogers, N.C., Schindler, C., Stark, G.R., Ihle, J.N., & Kerr, I.M. (1993). Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-gamma signal transduction pathway. Nature 366, 166-170.

Wen, Z., Zhong, Z., & Darnell, J.E. (1995). Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell 82, 241-250.

Wherry, J.C., Schreiber, R.D., & Unanue, E.R. (1991). Regulation of gamma interferon production by natural killer cells in *scid* mice: Roles of tumor necrosis factor and bacterial stimuli. Infect. Immun. 59, 1709-1715.

Wietzerbin, J., Gaudelet, C., Aguet, M., & Falcoff, E. (1986). Binding and cross-linking of recombinant mouse interferon-gamma to receptors in mouse leukemic L1210 cells; interferon-gamma internalization and receptor down-regulation. J. Immunol. 136, 2451-2455.

Wolf, S.F., Temple, P.A., Kobayashi, M., Young, D., Dicig, M., Lowe, L., Dzialo, R., Fitz, L., Ferenz, C., Hewick, R.M., Kelleher, K., Herrmann, S.H., Clark, S.C., Azzoni, L., Chan, S.H., Trinchieri, G., & Perussia, B. (1991). Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. J. Immunol. 146, 3074-3080.

Yphantis, D.A., & Arakawa, T. (1987). Sedimentation equilibrium measurements of recombinant DNA derived human interferon gamma. Biochemistry 26, 5422-5427.

CYTOKINES:

GRANULOCYTE COLONY-STIMULATING FACTOR

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I. INTRODUCTION

The colony-stimulating factors (CSFs) are a family of glycoproteins that are required for the survival, proliferation, and differentiation of hematopoietic precursor cells and the activation and survival of mature hematopoietic cells (Metcalf, 1986; Clark and Kamen, 1987; Morstyn and Burgess, 1988). The CSFs include granulocyte (G-) CSF, granulocyte-macrophage (GM-) CSF, macrophage (M-) CSF (CSF-1), and multi-CSF (interleukin-3). Together with erythropoietin, thrombopoietin, stem cell factor, and many of the interleukins, they are responsible for blood cell production from undifferentiated precursor cells in the bone marrow. The specificity of each of the CSFs for one or more different lineages of hematopoietic cells was first demonstrated in *in vitro* colony assays in semi-solid agar cultures (Metcalf, 1984). The

actions of G-CSF are largely restricted to cells of the granulocytic lineage.

G-CSF was first described as an activity that caused terminal differentiation of some myeloid leukemic cell lines (Burgess and Metcalf, 1980). Murine G-CSF was purified from conditioned medium made from the lungs of bacterial endotoxin treated mice (Nicola et al., 1983) and had a molecular weight of 25,000 kDa. Human G-CSF was subsequently purified from the conditioned medium of a bladder carcinoma cell line, 5637, (Welte et al., 1985) and a squamous cell carcinoma cell line, CHU-2, (Nomura et al., 1986). Both these cell lines constitutively secreted G-CSF of about 19,000 kDa molecular weight. The purified protein was partially sequenced, allowing the cloning of human G-CSF cDNAs (Nagata et al., 1986a; Souza et al., 1986). It was then possible to produce sufficient G-CSF for *in vivo* studies in animals and clinical trials in human subjects.

II. THE G-CSF GENE

A. Cloning of the G-CSF cDNA

Two human cDNAs were cloned independently from tumor cell lines using oligonucleotide probes based on the partial protein sequence of G-CSF (Nagata et al., 1986a; Souza et al., 1986). The cDNA sequences encoded mature proteins of 177 and 174 amino acid residues, respectively. Nagata later cloned a second cDNA encoding a predicted 174 residue protein and found that the shorter form had the higher biological activity (Nagata et al., 1986b). It is not known whether the longer form is produced by normal cells or is unique to carcinoma cell lines. A murine G-CSF cDNA was isolated by cross-hybridization with human cDNA under low stringency conditions (Tsuchiya et al., 1986). Only one murine cDNA was obtained, encoding a protein of 178 amino acid residues.

B. G-CSF Gene Structure

The chromosomal genes for human and murine G-CSF have been characterized (Nagata et al., 1986b; Tsuchiya et al., 1987). In each case there is a single gene copy and both genes have a similar structure of five exons and four introns. The exon sequences are highly conserved but there is little homology between the intron sequences. In the human gene,

there are two donor splice sites at the 5'-terminus of the second intron, allowing alternative splicing of the mRNA and accounting for the two cDNAs obtained from the CHU-2 cells. The murine gene does not contain the alternative splice site, thus alternative transcripts are not predicted. The human and murine genes have been mapped to homologous chromosomal locations. The human gene was localized to the q21-q22 region of chromosome 17 (Kanda et al., 1987; Simmers et al., 1987; Tweardy et al., 1987) and the murine gene was located on the distal half of chromosome 11 (Buchberg et al., 1988).

The sequences of the murine and human G-CSF gene promotor regions are highly conserved for up to 300 base pairs upstream from their transcription start sites (Tsuchiya et al., 1987). The promotor is responsive to tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), which induce G-CSF secretion from various cell types (see following), and contains at least three regulatory elements (Asano and Nagata, 1992; Shannon et al., 1992).

III. G-CSF PROTEIN STRUCTURE

The predominant form of human G-CSF contains 174 amino acid residues in the mature protein and shows a high degree of amino acid sequence identity with the murine protein of 178 amino acid residues (73%; Tsuchiya et al., 1986). Canine and bovine G-CSF have also been cloned and are both 175 residues long, with about 80% sequence identity with human G-CSF (Lovejoy et al., 1993). The high level of sequence homology correlates with a broader species specificity than is found with the other CSFs (Zsebo et al., 1986). G-CSF contains five cysteine residues. Lu et al. (1989) established that there are two disulphide bonds (Cys36-Cys42 and Cys64-Cys74) in human G-CSF and a free cysteine at position 17 that is inaccessible to solvent in the native molecule. The five cysteines are conserved in canine and bovine G-CSF, as are the four disulphide-bonded residues in the murine protein, but the fifth free residue is at position 89 in murine G-CSF.

Recombinant human G-CSF produced in *E. coli* has a molecular weight of 18.8 kDa, slightly less than the 19.6 kDa of G-CSF purified from mammalian cell supernatants (Souza et al., 1986). This difference was shown to be due to O-linked glycosylation, since it could be abolished by treatment with neuraminidase and O-glycanase (Souza et al., 1986). G-CSF does not contain any asparagine residues, thus there

are no potential N-linked glycosylation sites. Glycosylation does not affect the biological activity of G-CSF *in vitro*, but increases its stability, protecting it against denaturation and aggregation (Oheda et al., 1990). In human G-CSF, the glycosylation site is Thr133 (Kubota et al.,1990), and this residue is conserved in the other species.

The structure of human G-CSF was recently determined by X-ray crystallography (Hill et al., 1993; Figure 1). It is an antiparallel 4-α–helical bundle, as was predicted by Parry et al. (1988) and Bazan (1990) and is very similar to growth hormone (Abdel-Meguid et al., 1987; de Vos et al., 1992). Many of the cytokines have now been shown to have this general structure (Hill et al., 1993). The structure of bovine and canine G-CSF is almost identical to that of human G-CSF (Lovejoy et al., 1993), correlating with their inter-species biological crossreactivity. More recently nuclear magnetic resonance (NMR) spectroscopy has been used to analyze the solution structure of G-CSF, with similar results (Werner et al., 1994; Zink et al., 1994).

Both mutagenesis and mapping of monoclonal antibody binding sites have been used to identify regions of G-CSF that interact with its specific receptor. Kuga et al. (1989) tested the biological activity of a series of mutants. Many of these were internal deletions that abolished activity, but would be predicted to have major effects on structure that would lead indirectly to loss of function. However, deletion of 11 residues at the amino(N)-terminus did not affect function, indicating that the peptide preceeding the first α-helix does not interact with the receptor. It is interesting that deletion or substitution of Leu35 resulted in loss of activity because this is the site of insertion of three additional residues in the 177 residue protein, which also has reduced activity. This region near the carboxyl(C)-terminus of the first (A) helix is therefore likely to interact with the receptor.

An alternative approach was to map the binding site of neutralizing monoclonal anti-G-CSF antibodies (Layton et al., 1991). The binding of most of these antibodies was conformation-dependent, so digestion of unreduced G-CSF as well as peptide synthesis were used to localize antibody binding to residues 20-58. Now that G-CSF structure is known, this region can be seen to cover the C-terminal half of the A helix and the N-terminal half of the AB loop, including the short additional helix in this loop (Figure 1).

Lovejoy et al. (1993) have predicted which residues might interact with the receptor by identifying those that are conserved between species and are solvent accessible and also those that correspond to the receptor

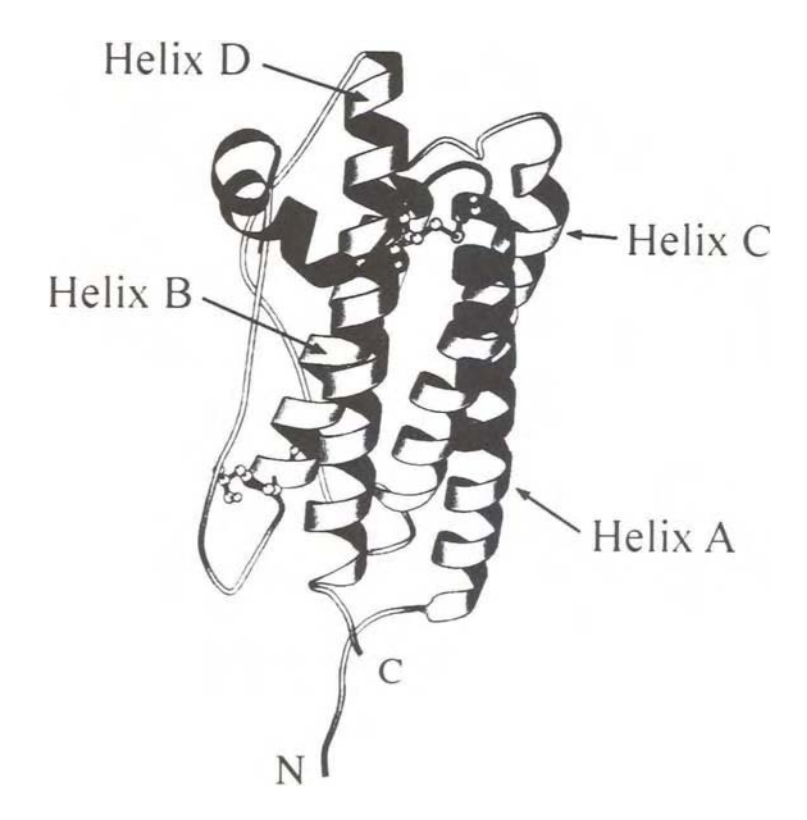


Figure 1. The three-dimensional structure of human G-CSF shown as a ribbon diagram. The region recognized by neutralizing antibodies is shown in black. The disulphide bonds are shown as ball and stick representations of the cysteine side chains.

binding residues of growth hormone. Many of the predicted residues are within the antibody binding site, which corresponds to part of receptor binding sites one and two of growth hormone (de Vos et al., 1992). More work is required to completely define the receptor binding site of G-CSF.

IV. THE G-CSF RECEPTOR (G-CSF-R)

The actions of G-CSF are mediated via direct interaction with a specific receptor (G-CSF-R). The G-CSF-R is a member of the hematopoietin receptor superfamily (Bazan, 1990). This diverse family includes the IL-6 receptor family, the IL-2 receptor family, GM-CSF, IL-3, and IL-5

receptor family, and erythropoietin and growth hormone receptors as well as the interferon and prolactin receptors (Thèze, 1994). These receptors can be divided into two classes. Those that share one or more receptor subunits such as the IL-2 receptor family and those that have only one unique receptor subunit. The G-CSF-R falls into this latter class. The G-CSF-R shares the greatest amino acid identity with gp130, the signaling chain of the IL-6 receptor family. The G-CSF-R is thought to dimerize following G-CSF binding (Fukunaga et al., 1990a, 1991; Horan et al., 1996) and in this respect is similar to the growth hormone receptor which is also activated by receptor homodimerization (Fuh et al., 1992). So far only one chain of the G-CSF-R has been described and this seems sufficient for high affinity binding and signal transduction in a variety of transfected cell lines (Fukunaga et al., 1991).

A. Receptor Expression

The G-CSF-R is a glycoprotein that is expressed almost exclusively on cells of the neutrophilic lineage and leukemic cell lines (Nicola and Metcalf, 1984, 1985). Receptor expression in murine bone marrow is restricted largely to granulocytic cells and appears to increase with cell development, with promyelocytes expressing about 150 receptors/cell and mature granulocytes expressing about 300 receptors/cell (Nicola, 1990). In human bone marrow, the promyelocytes expressed the highest receptor number (about 500) and mature neutrophils slightly fewer (Begley et al., 1988a), while peripheral blood neutrophils expressed about 1,000 receptors/cell (Nicola et al., 1986; Uzumaki et al., 1988). A subpopulation of $CD34^+$ progenitor cells expressed the G-CSF-R by fluorescence analysis (Shimoda et al., 1992). Expression of a high affinity receptor has also been detected on platelets with some indication that it may act to augment platelet aggregation (Shimoda et al., 1993).

The G-CSF-R has been detected on some non-hematopoietic cells. Bussolino et al. (1989) reported G-CSF-R expression on human endothelial cells, demonstrating G-CSF mediated migration and proliferation. In contrast, Yong et al. (1991) were unable to detect G-CSF-R expression on endothelial cells. The reason for this discrepancy is unknown. Receptors have also been reported on small cell lung cancer cell lines (Avalos et al., 1990) and human tonsillar B cells (Morikawa et al., 1993). The G-CSF-R is present on placental cells (Uzumaki et al., 1989), consistent with the cloning of the human G-CSF-R from a placental cDNA library (Larsen et al., 1990; Fukunaga et al., 1990c). Uzumaki et al. (1989) also

detected receptors on a trophoblastic cell line. The function of the G-CSF-R in non-hematopoietic cells is unclear, although it has been speculated to have a role in endothelial proliferation and migration (Bussolino et al., 1989).

Binding experiments with ^{125}I-labeled G-CSF detected a single, high affinity (60-300 pM) receptor on both normal and leukemic cells (Nicola and Metcalf, 1984; Uzumaki et al., 1988; Park et al., 1989; Avalos et al., 1990; Fukunaga et al., 1990a). Several receptor bands of molecular weight 110-150 kDa are seen by western blotting with specific antibodies or chemical cross-linking with radiolabeled G-CSF, presumably due to differing levels of glycosylation (Nicola and Peterson, 1986; Fukunaga et al., 1990c).

B. Receptor Structure

The human G-CSF receptor is predicted from its cDNA sequence to be 813 amino acids in length and consists of an extracellular domain, transmembrane domain and cytoplasmic domain containing 604, 26, and 183 amino acids, respectively (Fukunaga et al., 1990c; Figure 2). Four alternative transcripts have also been described (Fukunaga et al., 1990c; Larsen et al., 1990; Dong et al., 1995). There is considerable amino acid identity (62.5%) with the murine G-CSF-R which is an 812 amino acid polypeptide (Fukunaga et al., 1990b). Transfection of the receptor cDNA into myeloid cell lines can result in both a proliferative and differentiative response to G-CSF (Fukunaga et al., 1991).

The *extracellular domain* has an immunoglobulin-like structure followed by a 200 amino acid region, the cytokine receptor homologous region (CRHR) or hematopoietin domain. The CRHR was originally identified as a conserved region of homology within the cytokine receptor family (Bazan, 1990). It is characterized by four conserved cysteines in the amino-terminal region and a Trp Ser X Trp Ser motif (where X is a non-conserved amino acid) in the C-terminal region (Fukunaga et al., 1990b, 1990c; Larsen et al., 1990). The structure of the G-CSF-R CRHR is predicted to be similar to that of the growth hormone receptor. Consisting of two 100 amino acid regions linked by a short polypeptide chain, each region contains seven β strands divided into two sheets (de Vos et al., 1992). Recent work by Hiraoka et al. (1994) using NMR analysis of the solublized amino-terminal region of the CRHR supports this prediction. The conserved cysteines in the amino-terminal of the CRHR form disulphide bonds which are critical

for maintaining a stably folded protein (Hiraoka et al., 1994). The CRHR is followed by three repeats of a fibronectin type III domain and the transmembrane domain (Fukunaga et al., 1990b, 1990c; Larsen et al., 1990; Figure 2).

Studies with deletion mutants and soluble receptors have demonstrated that the amino-terminal region of the CRHR is absolutely required for ligand binding with a contribution by the C-terminal region to high affinity binding (Fukunaga et al., 1991; Hiraoka et al., 1994). Deletion of the amino-terminal region of the CRHR eliminates both ligand binding and the proliferative response. Deletion of the C-terminal region of the CRHR reduces binding affinity and rather suprisingly, completely eliminates proliferation. This suggests that the carboxyl region is involved in receptor dimerization or is conformationally required for cytoplasmic activation (Fukunaga et al., 1991).

The immunoglobulin-like domain is required for dimerization (Hiraoke et al., 1996). The function of the fibronectin repeats is unknown but they may contribute to receptor stability (Fukunaga et al., 1991).

Three regions (boxes) of homology have been identified within the *cytoplasmic domains* of the hematopoietin receptor superfamily (O'Neal and Yu-Lee, 1993). The Box 1 region lies in the membrane proximal part of the cytoplasmic domain and is characterized by a conserved proline

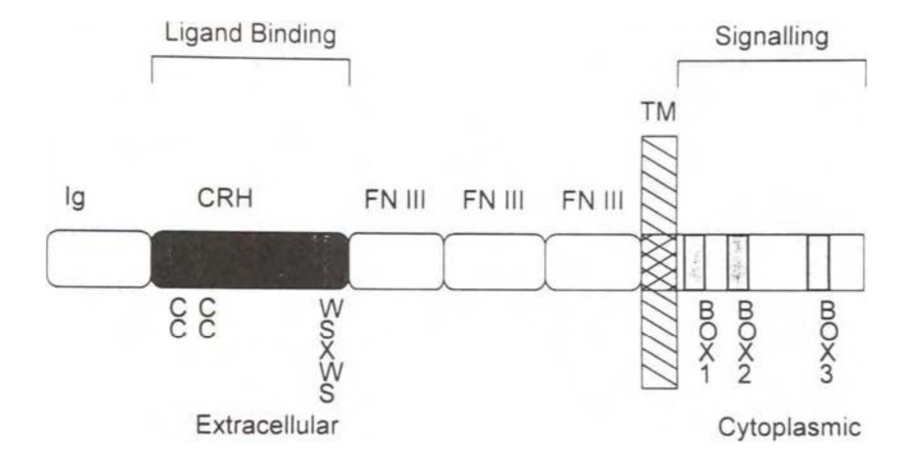


Figure 2. Diagram of the G-CSF-R showing the domain structure and regions of functional importance. Ig, immunoglobulin; CRH, cytokine receptor homology; FN III, fibronectin type three; TM, transmembrane; C, conserved cysteine residue; WSXWS, conserved amino acid sequence

motif. The consensus sequence for the G-CSF-R and the Box 1 regions of the other cytokine receptors can be condensed to Ψ X X X Ala Pro X Pro, where Ψ refers to a hydrophobic residue and X is any amino acid (O'Neal and Yu-Lee, 1993).

Box 2 is an acidic region sharing loose homology within the hematopoietin receptor superfamily. Box 3 homology is shared only between the G-CSF-R and gp130 and is situated toward the C-terminus of the cytoplasmic domain (Fukunaga et al., 1991).

Deletion of the cytoplasmic domain does not affect G-CSF binding but abrogates the proliferative response to ligand (Fukunaga et al., 1991). The first 57 amino acids of the G-CSF-R cytoplasmic domain containing the Box 1 and Box 2 regions are critical for transmission of the proliferative signal, although for a full proliferative response 100 amino acids seem to be required (Ziegler et al., 1993; Fukunaga et al., 1991). The Box 1 region is itself critical for proliferation. Fukunaga et al. (1993) elegantly demonstrated this by showing that mutation of the Box 1 proline residues to alanine was sufficient to disrupt the proliferative response.

The C-terminal region of the cytoplasmic domain is involved in differentiation. Fukunaga et al. (1993) reported an increase in myeloperoxidase (MPO) and leukocyte elastase gene expression in myeloid cell lines in response to G-CSF. The C-terminal region of the cytoplasmic domain was necessary for induction of the MPO response. Interestingly, the Box 1 domain was also required. This again suggests a critical role for the Box 1 domain in receptor signaling and it can be hypothesized that the MPO response is mediated by a kinase associated with the Box 1 region which phosphorylates the C-terminal region (Fukunaga et al., 1993).

Truncation of the C-terminal 98 amino acids resulted in the failure of G-CSF to induce neutrophilic maturation in L-GM, a murine myeloid cell line (Dong et al., 1993). The presence of this region also inhibited the proliferative response in some cell types, suggesting that it mediates negative regulatory effects (Dong et al., 1993).

C. Signal Transduction

G-CSF interaction with the receptor ultimately results in the proliferation of granulocytic precursors and their terminal differentiation into mature neutrophils. It also enhances a variety of neutrophil functions including survival, phagocytosis and superoxide production. How these

diverse signals are transmitted from the receptor has been a subject of great interest.

Until recently very little was known about the molecules which interact with the G-CSF-R to transduce signals from the receptor to the nucleus. Tyrosine phosphorylation of proteins in response to G-CSF has been reported by several groups (Evans et al., 1990; Isfort and Ihle, 1990; Demetri and Griffin, 1991). Over the last few years some of these proteins have been identified (Figure 3).

Like many other members of the hematopoietin receptor superfamily, the G-CSF receptor is tyrosine phosphorylated after ligand binding (Pan, et al., 1993; Nicholson et al., 1994) on one or more of the four candidate tyrosines situated in the C-terminal region of the human G-CSF-R. As the carboxyl region containing the tyrosines can be deleted without

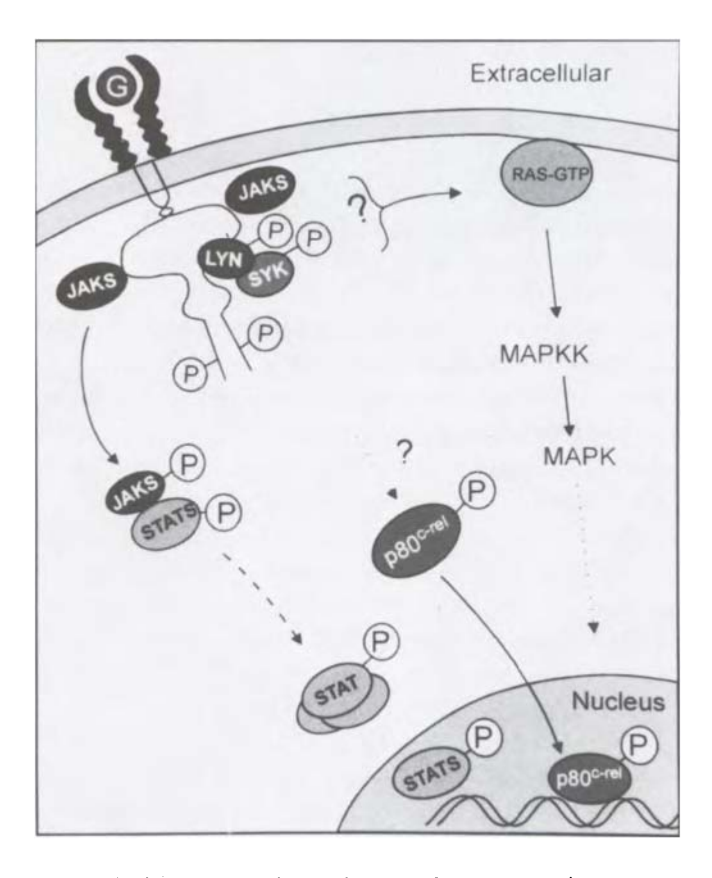


Figure 3. Model of G-CSF signal transduction, showing cytoplasmic proteins that have been implicated in early responses to G-CSF.

abrogating the proliferative response it has been suggested that tyrosine phosphorylation of the receptor is not required for proliferation (Pan et al., 1993). Recently, several of the tyrosine residues have been shown to be crucial for G-CSF-induced differentiation (Yoshikawa et al., 1995; Nicholson et al., 1996).

It is thought that the phosphorylated tyrosines may act to recruit a second wave of SH2-containing signaling molecules. SH2 or Src Homology 2 refers to a conserved 100 amino acid stretch within proteins which binds with high affinity to phosphotyrosines (Montminy, 1993). It is not yet known which kinase is responsible for tyrosine phosphorylation of the receptor although the JAK family of protein tyrosine kinases have emerged as strong contenders.

The JAK kinases, originally named as "Just Another Kinase," alias *janus kinase* in reference to the tandem kinase domains, are involved in signaling from many of the hematopoietin receptors (Wilks and Harpur, 1994; Ihle et al., 1994). G-CSF induces the tyrosine phosphorylation and kinase activation of both JAK1 and JAK2 (Nicholson et al., 1994; Tian et al., 1994). JAK1 has been shown to co-immunoprecipitate with the G-CSF-R and appears to associate with the receptor both before and after ligand binding (Nicholson et al., 1994).

In gp130, the IL-6 signaling component, mutation of the prolines in Box 1 or the deletion of Box 2 results in a loss of both IL-6 mediated proliferation and JAK2 tyrosine phosphorylation (Narazaki et al., 1994). JAK2 has been shown to interact with the Box 1 region of the erythropoietin receptor (Witthuhn et al., 1993). G-CSF activation of JAK2 requires a region of the receptor encompassing Box 1 and Box 2, while Box 1 alone is not sufficient for JAK2 activity (Nicholson et al., 1995). It is not yet known whether the conserved proline residues mediate receptor interaction with the JAK kinases or whether the prolines are structurally required for cytoplasmic activation following dimerization of the receptor. The two may not be mutually exclusive.

The JAK kinases are hypothesized to tyrosine phosphorylate STAT (signal transducers and activators of transcription) molecules which are then able to translocate to the nucleus (Shuai et al ., 1993; Darnell et al., 1994). STAT3 has been identified as the main member of the STAT family involved in G-CSF signaling although there is some indication that STAT1 may also be involved, perhaps acting as a heterodimer with STAT3 (Tian et al., 1994). Stat3 activation appears to require the carboxyl terminus of the G-CSF-R (Nicholson et al., 1995; de Koning et al., 1996).

Other kinases have been shown to interact with the G-CSF-R and become active in response to G-CSF. Lyn, a member of the Src family of tyrosine kinases was shown to be activated in a murine cell line and in human neutrophils in response to G-CSF. It was shown to be constitutively associated with the G-CSF-R (Corey et al., 1994). Corey et al. (1994) also described Syk kinase activation and the formation of a Lyn/Syk/G-CSF-R complex following ligand activation of the receptor. However, Lyn kinase activation in response to G-CSF does not appear to be consistent. Torigoe et al. (1992) found no evidence of Lyn kinase induction in a human leukemic cell line. However, these are only two reports and the differences may simply reflect cell line or species differences in the ability of the G-CSF-R to utilize available signaling pathways. Alternatively, the differences may reflect the different biological responses elicited by G-CSF *in vivo.*

Ras activity and mitogen-activated protein kinase (MAPK) activity have also been reported in response to G-CSF with some evidence that the $p21^{ras}$/MAPK pathway is responsible for transducing the proliferative response (Bashey et al., 1994). What is not yet known is the link between the receptor and the $p21^{ras}$ pathway although it seems likely that the adaptor protein Shc may constitute part of that link. Shc is an SH2 domain-containing protein which has been shown to be tyrosine-phosphorylated in response to other cytokines (IL-3, erythropoietin, Steel factor, IL-2, GM-CSF, M-CSF; Cutler et al., 1993; Ravichandran and Burakoff, 1994; Welham et al., 1994) and becomes associated with the adaptor protein Grb2 and the guanine nucleotide exchange protein Sos1 (Cutler et al., 1993; Welham et al., 1994).

Tyrosine-phosphorylation and increased DNA binding of the transcription factor $p80^{c\text{-}rel}$ in response to G-CSF has been described in human neutrophils (Druker et al., 1994). The $p80^{c\text{-}rel}$ protein is a proto-oncogene related to the NF-kB family. The functional significance of $p80^{c\text{-}rel}$ in human neutrophils has yet to be determined (Druker et al., 1994).

Very little is known about gene transcription initiated by G-CSF stimulation of cells. In myeloid cells the early response genes junB and c-fos but not c-jun are transcribed following G-CSF activation, paralleling induction of cell proliferation (Adachi and Saito, 1992). GIG1, a novel G-CSF-induced gene has been identified by Shimane et al. (1994). GIG1 mRNA was detected in myeloid cell lines but not in erythroid or lymphoid cell lines and is predicted to encode a 167 amino acid mem-

brane protein (Shimane et al., 1994).

It is still not clear which biochemical pathways are activated by the G-CSF-R to result in the diverse physiological effects elicited by G-CSF. Clearly several different pathways are activated, potentially with the JAK kinases initiating a cascade of phosphorylation events. It seems likely that novel pathways and signaling molecules are yet to be discovered to add to those discussed here.

V. PRODUCTION OF G-CSF

Many different cell types have been shown to produce G-CSF in in vitro culture systems (reviewed in detail by Sallerfors, 1994), most commonly in response to cytokines or bacterial products, but there is some evidence of constitutive production. The interpretation of reports of constitutive production is difficult because the manipulation of cells that is required to establish relatively pure cell populations may be sufficient to activate G-CSF production. In addition, the use of highly sensitive techniques such as RT-PCR (reverse transcription of mRNA followed by amplification of the cDNA of interest by the polymerase chain reaction) may detect levels of mRNA that are not biologically significant. Whether G-CSF is produced constitutively *in vivo* cannot be established from these experiments. Nevertheless, it is clear that G-CSF production is inducible in cell types that are widely distributed throughout the body.

Bone marrow is the site of hematopoiesis in adults and some time ago, Dexter et al. (1977) described a murine bone marrow culture system that maintained long-term hematopoiesis *in vitro*, in the presence of an adherent stromal layer. Since then, a considerable effort has been made to identify the cytokines that are produced by the heterogeneous stromal cells, but CSF production has been difficult to demonstrate (Shadduck et al., 1983). Kittler et al. (1992) detected constitutive production of G-CSF mRNA that was increased in response to IL-1 or pokeweed mitogen stimulation, although they did not determine whether these mRNA levels correlated with protein production. Bone marrow-derived macrophages produced G-CSF after induction with pokeweed mitogen and CSF-1 (M-CSF; Temeles et al., 1993). Other groups have analyzed CSF production from cloned cell lines derived from marrow stroma. Rennick et al. (1987) detected constitutive G-CSF production from a stromal cell clone that was substantially increased by LPS or IL-1

stimulation. More recently, a different stromal cell line has been shown to produce G-CSF in response to adhesion of a murine myeloid leukemic cell line (NFS-60), suggesting that adhesion of progenitor cells to bone marrow stroma may activate G-CSF production in normal marrow (Yoshikubo et al., 1994).

Human long-term bone marrow cultures have also been studied for cytokine production. Individual stromal cell layers have shown heterogeneity in their constitutive production of G-CSF, but all appear to produce G-CSF after IL-1 stimulation (Fibbe et al., 1988a; Yang et al., 1988; Migliaccio et al., 1992a). The apparent constitutive production may be in response to endogenous IL-1, since Fibbe et al. (1988a) found that it was inhibited by anti-IL-1 antiserum. Isolated bone marrow macrophages also produce G-CSF in response to IL-1 (Fibbe et al., 1986). Cloned cell lines were difficult to establish from human stroma, but several groups were successful using SV40 viral transformation (Nemunaitis et al., 1989; Novotny et al., 1990). The clones showed heterogeneity in G-CSF production but were all able to support bone marrow colony growth (Novotny et al., 1990) or cord blood progenitor cell proliferation (Cicuttini et al., 1992). These results seem to suggest that G-CSF is not required for progenitor cell proliferation, but might also be explained if levels of G-CSF below the detection limit of the assay used were active. Another possibility is that G-CSF remains bound to the surface of some stromal cell lines, as has been demonstrated for cultured human osteoblasts (Taichman and Emerson, 1994). The osteoblasts constitutively produced G-CSF that was not detectable in culture supernatants but contributed to the growth of CD^{34+} bone marrow stem cells, as shown by anti-G-CSF antibody inhibition of stem cell growth. Thus, osteoblasts may be an important source of constitutive G-CSF for steady-state granulopoiesis.

Monocytes, fibroblasts, and endothelial cells from sources outside the bone marrow can also be induced to secrete G-CSF by bacterial products and inflammatory mediators. Adherent monocytes derived from human peripheral blood are stimulated by lipopolysaccharide (LPS; Vellenga et al., 1988), IL-1 (Fibbe et al., 1986), GM-CSF and IL-3 (Oster et al., 1989), and to a small extent, TNF-α (Sallerfors and Olofsson, 1992) to secrete G-CSF. Increased G-CSF expression is mediated mostly by increased message stability rather than increased message transcription (Ernst et al., 1989). Reports of the effects of interferon-γ (IFN-γ) on G-CSF production are mixed, with some groups finding a positive effect (Oster et al., 1989), while others found no effect (Hamilton et al., 1992a;

de Wit et al., 1993). However, the latter two studies found that IFN-γ increases the effect of LPS. The augmented response to LPS was the result of a small increase in the rate of G-CSF mRNA transcription and a greater effect on mRNA stability (de Wit et al., 1993). Less is known about factors that inhibit G-CSF secretion. Two cytokines with anti-inflammatory activities, IL-4 and IL-10, have been shown to suppress induced G-CSF production (de Waal Malefyt et al., 1991; Hamilton et al., 1992a).

Fibroblasts from various sources secrete G-CSF after stimulation with IL-1 (Fibbe et al., 1988b; Kaushansky et al., 1988) or TNF-α (Koeffler et al., 1987; Leizer et al., 1990). TNF-α increased the stability of G-CSF mRNA (Koeffler et al., 1988). In contrast with their effects on monocytes, IL-4 augments the stimulatory effect of IL-1 on G-CSF production by fibroblasts and interferon-γ is suppressive (Tushinski et al., 1991; Hamilton et al., 1992b).

Vascular endothelial cells produce G-CSF in response to LPS and IL-1 (Broudy et al., 1987; Seelentag et al., 1987; Zsebo et al., 1988), but data about the effect of TNF-α is contradictory (Broudy et al., 1986; Seelentag et al., 1987; Brown et al., 1993). IL-4 had little effect on IL-1-stimulated

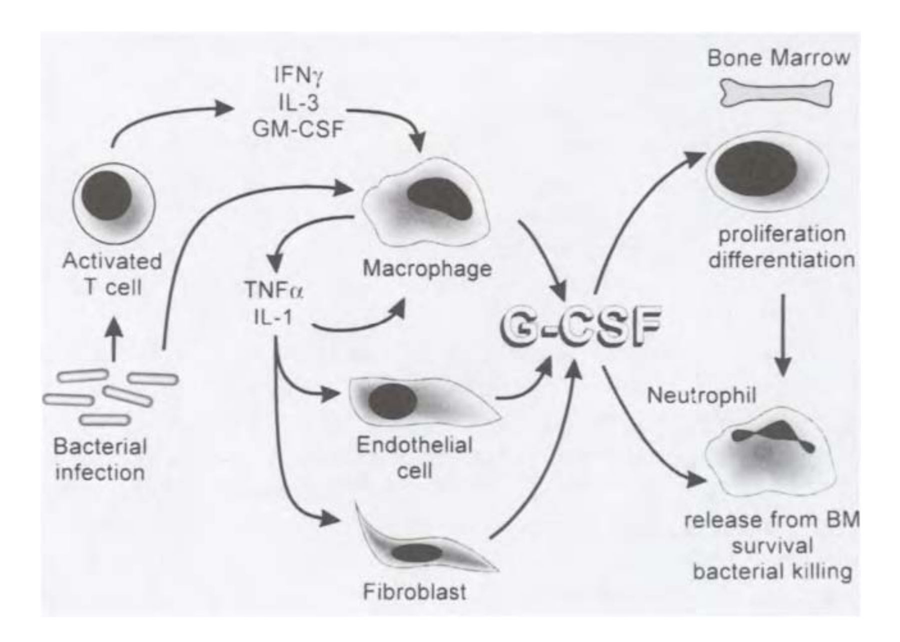


Figure 4. Schematic representation of the sources and actions of G-CSF (simplified).

G-CSF synthesis (Zoellner et al., 1993). Oncostatin M, a member of the hematopoietic cytokine family, induces G-CSF secretion (Brown et al., 1993).

Other cell types that can be induced to produce G-CSF include astrocytes (Malipiero et al., 1990; Tweardy et al., 1991; Aloisi et al., 1992; Stan et al., 1994), peritoneal mesothelial cells (Lanfrancone et al., 1992), arterial smooth muscle cells (Zoellner et al., 1992), articular cartilage and chondrocytes (Campbell et al., 1991), and germinal centre B lymphocytes (Corcione et al., 1994).

These *in vitro* studies provide good evidence that the body is well equipped to respond to local infection or other source of inflammation by stimulation of local production of G-CSF and recruitment and activation of neutrophils (Figure 4). However, they are unable to establish whether there is constitutive production of G-CSF *in vivo* and whether it is required for steady state granulopoiesis. There is now evidence from other studies that G-CSF is required for normal neutrophil production as well as optimal response to infection (see following).

VI. BIOLOGICAL EFFECTS OF G-CSF

A. *In Vitro* Activities of G-CSF

Early studies of the actions of CSFs were of necessity carried out with impure preparations of CSF and heterogeneous populations of responding cells (mostly unfractionated bone marrow). Thus, interpretation of these data could always be criticized because other factors either in the impure CSF sources or produced by the mixed responding cell populations might have contributed to the observed effects. More recently, the cloning of the CSF cDNAs and identification of surface markers on progenitor cells has allowed these studies to be repeated and extended with purified CSFs and much more highly purified populations of progenitor cells (reviewed by Visser and Van Bekkum, 1990). It is gratifying that most of the early results have been confirmed by the more definitive recent studies.

G-CSF promotes the proliferation and differentiation of progenitor cells in the neutrophil lineage and costimulates mature neutrophil functions (reviewed by Nicola, 1990; Demetri and Griffin, 1991). The survival of precursors of other lineages is enhanced by G-CSF without

any apparent proliferation (Metcalf and Nicola, 1983). Relatively mature progenitor cells in bone marrow (colony-forming cells) respond to G-CSF in semi-solid agar cultures, forming predominantly granulocytic colonies in 7-14 days (Metcalf and Nicola, 1983; Welte et al., 1985; Nomura et al., 1986; Souza et al., 1986). At high G-CSF concentrations, some macrophages are also found. The human progenitor population defined by expression of the CD34 antigen (Baines et al., 1988; Baum et al., 1992) can be subdivided into CD33 $^+$ and CD33- cells. The more primitive CD33- cells did not respond to G-CSF whereas the CD33$^+$ progenitors did respond (Ema et al., 1990). G-CSF is required continuously for colony formation since promyelocytes and myelocytes stimulated with a single pulse of G-CSF gave rise to clones of only two cells in most cases and blast cells (more primitive progenitors) were unable to divide after a single pulse (Begley et al., 1988b).

G-CSF can also interact with other cytokines to give synergistic responses, resulting in increased colony numbers and/or colony size. The increase in colony numbers is due to an increase in colonies containing mixed cell lineages. Thus, G-CSF can contribute to the production of cells other than granulocytes when other cytokines are present. For example, G-CSF has synergistic activity with GM-CSF (McNiece et al., 1989; Schaafsma et al., 1989), IL-3 (Ikebuchi et al., 1988), or with combinations of two other CSFs (Metcalf and Nicola, 1992). It also synergizes with *kit* ligand (also known as steel factor, stem cell factor, and mast cell growth factor; Martin et al., 1990; Bernstein et al., 1991; McNiece et al., 1991; Tsuji et al., 1992), IL-1 (Schaafsma et al., 1989), IL-7 (Jacobsen et al., 1994), and IL-6 (Rennick et al., 1989), although others have found that IL-6 inhibits G-CSF activity (Katayama et al., 1990; Pojda et al., 1992). IL-4 appears to be unusual because it interacts with G-CSF to increase the production of neutrophils, but not cells of other lineages (Peschel et al., 1987; Sonoda et al., 1990). IFN-g inhibits G-CSF-stimulated production of granulocytic colonies (Koike et al., 1992) but increases monocytic colonies and this effect is opposed by IL-4 (Snoek et al., 1993).

The molecular mechanisms involved in synergistic effects have not been well studied. The limited data available suggest that several mechanisms may operate. Flow cytometric analysis of human CD34$^+$ progenitors showed that the combination of G-CSF and GM-CSF accelerated transit through the cell cycle (Lardon et al., 1993). Other studies show that G-CSF can upregulate expression of receptors for synergizing

factors, for example, the IL-1 receptor (Dubois et al., 1992) and the IL-3 receptor α-chain (Sato et al., 1993).

More primitive progenitors that give rise to colony-forming cells when grown in liquid culture in the presence of cytokines are also present in the $CD34^+$ (human) or $Sca1^+$ Lin^- (murine; Spangrude et al., 1988) populations. G-CSF has little effect on these early cells when used alone, but can synergize with other factors such as *kit* ligand, IL-1, IL-6, and other CSFs to expand the production of colony-forming cells (Bodine et al., 1991; Haylock et al., 1992; Migliaccio et al., 1992b; Muench et al., 1992; Lu et el., 1993; Mayani et al., 1993). An even earlier population has been studied by the use of sequential two-stage cultures followed by colony forming cell assays. Maintenance of these long-term culture-initiating cells may, but need not, include G-CSF (Sutherland et al., 1993).

B. Differentiation

CSFs are thought to be required for both proliferation and differentiation of hematopoietic precursors. However, because these two processes are occurring simultaneously, it is difficult to study each one separately. Differentiation of neutrophils appears to require the shutdown of proliferation and activation of genes required for mature cell functions. Some investigators believe that differentiation is a stochastic process and that the only role of CSFs is to promote survival and proliferation (Ogawa, 1993), while others believe that the CSFs are required to direct differentiation (Metcalf, 1991). In support of the stochastic theory, Fairbairn et al. (1993) have shown that transfection of the *bcl-2* gene into an IL-3-dependent cell line (FDCP-Mix) suppressed the apoptosis (programmed cell death) that normally occurs on withdrawal of IL-3 and allowed differentiation of the cells in the absence of CSFs and in the absence of proliferation. Other experiments support a direct influence of CSFs on differentiation. For example, Fukunaga et al. (1993) and Dong et al. (1993) have identified a region of the G-CSF receptor that is required for transduction of differentiative signals in response to G-CSF and Shimane et al. (1994) have characterized a gene induced by G-CSF. Although several leukemic cell lines differentiate in response to G-CSF (Begley et al., 1987a; Li et al., 1993), it has been claimed that G-CSF is required for survival of mature progeny rather than for differentiation (Böhmer and Burgess, 1988). Further work will be needed to determine to what extent G-CSF directly affects differentiation.

C. Activity on Mature Cells

G-CSF enhances the survival of human neutrophils (Begley et al., 1986) by preventing apoptosis (Williams et al., 1990). In contrast, a separate study found that G-CSF had only a slight effect on survival, but GM-CSF was very effective (Brach et al., 1992). It is not clear why these studies are in disagreement, but it is possibly because different culture conditions were used.

G-CSF activates or primes neutrophil functions although usually to a lesser extent than GM-CSF. The neutrophil respiratory burst in response to stimuli such as the bacterial peptide f-Met-Leu-Phe is primed by G-CSF (Kitagawa et al., 1987; Nathan, 1989; Sullivan et al., 1993) but the effect is relatively small and is strongly enhanced by TNFα (Khwaja et al., 1992). G-CSF enhances phagocytosis of bacteria (Roilides et al., 1991) and yeast (Yamamoto et al., 1993), and improves the defective function of neutrophils from patients infected with HIV-1 (Roilides et al., 1991) or chinchillas infected with influenza A viruses (Abramson and Hudnor, 1994). However, in the chinchilla model, *in vivo* G-CSF treatment did not reduce the incidence of secondary pneumoccocal disease. Antibody-dependent cell-mediated cytotoxicity of neutrophils for tumor cells was enhanced by G-CSF (Lopez et al., 1983; Vadas et al., 1983; Baldwin et al., 1993).

G-CSF may be involved in the recruitment of neutrophils to sites of inflammation because of its ability to alter neutrophil adhesion properties (Okada et al., 1990; Yuo et al., 1990) and its chemotactic activity (Wang et al., 1988). Although G-CSF alters the expression of neutrophil surface adhesion molecules such as CD11b/CD18 and LAM-1 (leukocyte adhesion molecule-1), these changes did not affect adhesion to endothelial cells (Yong and Linch, 1992). The effect of G-CSF on transendothelial migration was small in comparison to the effect of IL-8 (Smith et al., 1994), suggesting little role for G-CSF. Nevertheless, it may be of some importance because *in vivo* administration of G-CSF resulted in a rapid transient reduction in circulating neutrophils, possibly due to extravazation (Morstyn et al., 1988).

D. Activity on Non-myeloid Cells

There are a few reports that G-CSF has activity on some non-myeloid cell types. Hirayama et al. (1992) found that G-CSF in combination with *kit*-ligand supported the growth of murine B lymphocyte precur-

sors in a clonal assay. In a different system where progenitors were grown on stromal cells, G-CSF inhibited the differentiation of pre-B cells (Lee et al., 1993), indicating that the effect of G-CSF may depend on the stage of B cell maturation or the presence of other cytokines. Activated, mature human B cells increased their level of immunoglobulin secretion by three to fourfold in response to G-CSF (Morikawa et al., 1993).

One group has reported that human endothelial cells proliferate and migrate in response to G-CSF (Bussolino et al., 1991), but others have been unable to confirm these results (Yong et al., 1991). Human aortic smooth muscle cells and lung fibroblasts have also been reported to migrate in response to G-CSF (Valliant et al., 1993). Thus, G-CSF may be involved in tissue repair and fibrosis.

E. Physiological Role of G-CSF

The above *in vitro* studies implicate G-CSF in the maintenance of steady state hematopoiesis and in "emergency" responses to infection and inflammation. There is other indirect evidence to support these roles of G-CSF. When homeostasis is perturbed by diseases such as congenital neutropenia (Mempel et al., 1991) or infection (Watari et al., 1989), or in neutropenia following chemotherapy (Baiocchi et al., 1993), serum levels of G-CSF increase and in some situations have been shown to be inversely proportional to the circulating neutrophil level (see following). These observations suggest that G-CSF is produced to increase or restore neutrophil levels *in vivo.*

The development of neutralizing anti-G-CSF antibodies in dogs being treated with human G-CSF led to chronic neutropenia in these animals until the antibody titre declined (Hammond et al., 1991). This study provided the first more direct evidence of the importance of G-CSF for normal neutrophil production. However, the neutropenia was not absolute and bone marrow neutrophil precursors were not severely depleted. It is not clear whether the residual neutrophil production was due to incomplete G-CSF blockade or the activity of other cytokines. An elegant study designed to determine the normal physiological role of G-CSF was that of Lieschke et al. (1994), in which G-CSF "knockout" mice were generated from embryonal stem cells in which the G-CSF gene was disrupted. Peripheral blood neutrophil levels were reduced to 20-30% of normal in the deficient mice, bone marrow progenitors were reduced and control of infection was also markedly impaired. The neutropenia in the mice was not as profound as that seen in the dogs

described above, suggesting that there may be species variation in the degree to which granulopoiesis is dependent on G-CSF. Alternatively, there may have been some antibody-mediated removal of neutrophils in the dogs, as suggested by Hammond et al. (1991).

In humans, G-CSF may play an even more critical role in regulating neutrophil production. Recently it has been established that a child with severe congenital neutropenia had impaired G-CSF receptor function as a result of a mutation in one allele of the receptor (Dong et al., 1994). Children with this disease suffer from chronic neutropenia and are prone to spontaneous life threatening infections. This is a more pronounced phenotype than the G-CSF deficient mice which only demonstrated increased susceptibility to infection when specifically challenged with a pathogen.

F. Pharmacology of G-CSF

Administration of G-CSF to normal animals resulted in a rapid, dose-dependent elevation of circulating neutrophils (Cohen et al., 1987; Tamura et al., 1987; Welte et al., 1987; Ulich et al., 1988). Neutrophil levels returned to normal within 24-48 h of cessation of G-CSF, consistent with a direct effect of G-CSF on neutrophil production. Long-term exposure of mice to high circulating G-CSF levels, achieved by transplantation with marrow cells infected with a retroviral vector containing a G-CSF cDNA insert, resulted in a dramatic neutrophilia but no evidence of tissue damage (Chang et al., 1989). In normal human volunteers, similar elevations of neutrophil levels after subcutaneous G-CSF administration were reported (Bensinger et al., 1993; Caspar et al., 1993; Chatta et al., 1994). In most studies, the circulating neutrophils were found to function normally, but a recent report describes neutrophil activation (degranulation) after G-CSF administration in humans (de Haas et al., 1994).

Mobilization of progenitor cells into the peripheral blood after G-CSF injection was first described in an early clinical trial (Dührsen et al., 1988) and has also been described in mice (Neben et al., 1993). The cell surface adhesion molecule, VLA4, has been implicated in this mobilization, since treatment of primates with anti-VLA4 antibody caused progenitor mobilization and enhanced G-CSF-induced mobilization (Papayannopoulou and Nakamoto, 1993).

The effect of G-CSF on the kinetics of granulopoiesis in mice and humans has been studied using radioactive labeling techniques (Lord et

al., 1989, 1991). The marrow transit time for maturing granulocytes was substantially reduced and extra amplification divisions were calculated to occur, but there was no effect on the half-life of circulating neutrophils.

VII. PATHOPHYSIOLOGY OF G-CSF

A. Neutropenic States—The Relationship Between G-CSF Levels and Neutrophil Mass

To date, G-CSF deficiency states have not been documented in clinical practice. Five patients with severe congenital neutropenia (SCN) had G-CSF and GM-CSF mRNA in monocytes, suggesting that deficiency of these CSFs was not responsible for SCN (Bernhardt et al., 1993). Indeed, it generally appears that an inverse relationship exists between serum G-CSF levels and neutrophil counts in aplastic anemia, cyclic neutropenia, and after induction chemotherapy for leukemia or marrow transplantation (Watari et al., 1989; Sallerfors and Olofsson, 1991). These observations suggest that G-CSF plays a regulatory role in the body's attempts to increase neutrophil production. In some of the cases cited there was a clear relationship between G-CSF peak levels and infective episodes (Sallerfors and Olofsson, 1991) and it seems likely that exposure to infection or bacterial products during neutropenic nadirs stimulated increased cytokine release. In our own studies, neutropenia and infection were independent variables associated with G-CSF elevation (Cebon et al., 1994). The inverse relationship between serum levels of G-CSF and neutrophil counts has also been demonstrated in pharmacodynamic studies. The increasing neutrophil mass may contribute to increased G-CSF clearance through specific receptors (Layton et al., 1989).

B. Infection

The major stimulus for G-CSF release into the circulation *in vivo* appears to be infection. Bacterial products such as lipopolysaccharide stimulate the release of circulating colony-stimulating factors (Golde and Cline, 1975; Cheers et al., 1988). Specific immunoassays have been used to identify some of the mediators of the host response to infection, including G-CSF (Watari et al., 1989; Kawakami et al., 1990; Gessler et al., 1993; Cebon et al., 1994). GM-CSF, M-CSF, IL-6, IL-1, and IL-8

are also elevated (Casey et al., 1993; Cebon et al., 1994). These studies implicate G-CSF as one of the mediators of the leukocyte response which accompanies infection.

C. Febrile Neutropenia

In a survey of febrile episodes in neutropenic and non-neutropenic patients, G-CSF levels varied over a range of more than three orders of magnitude (from less than 100 pg/ml to greater than 100 ng/ml). The kinetics of the G-CSF response were very similar to those of IL-6 which also varied in concentration between patients (Cebon et al., 1994). Part of the considerable inter-patient variability was attributed to the severity of infection and pathogen type. For G-CSF, the neutrophil count was also important. Indeed, G-CSF was always detectable in neutropenic patients with proven infections but undetectable in some patients with documented bacteremia and normal neutrophil counts.

In contrast GM-CSF was generally not elevated in these febrile patients, although there are anecdotal reports of elevated levels in some patients with overwhelming sepsis. Levels do not appear to be affected by the neutrophil count and GM-CSF may function predominantly in the tissues.

D. Myeloablation and Marrow Transplantation

The elevation of cytokine levels following a myelotoxic or myeloablative insult implies a regulatory role during marrow recovery. Analysis may, however, be complicated by the effects which concurrent events such as infection, mucositis, tissue damage, transfusion, and graft-versus-host disease might have on cytokine levels. A number of investigators have detected elevated serum G-CSF, GM-CSF, IL-3, IL-6, IL-1α, M-CSF, and IL-8 (Beksac et al., 1992; Baiocchi et al., 1993; Haas et al., 1993; Kawano et al., 1993; Mangan et al., 1993; Rabinowitz et al., 1993) following myeloablation and marrow or peripheral blood progenitor cell transplantation. The inverse relationship between G-CSF levels and neutrophil counts after myeloablative therapy has been confirmed (Haas et al., 1993; Kawano et al., 1993; Sallerfors and Olofsson, 1991), and was also described for IL-3 (Mangan et al., 1993). Time course studies have demonstrated kinetic profiles which imply a relationship between cytokine levels and engraftment and it has been proposed that cytokines are secreted coordinately for the production and activation of neutro-

phils. However, when a clinically complex situation such as marrow transplantation is studied, many other events are occuring which may influence cytokine release and measurable cytokine levels may be altered by each of these. Attributing cause and effect relationships between endogenous cytokine responses and clinical endpoints therefore needs to be undertaken cautiously.

E. Neonatal Neutrophil Production

Neonates, especially when premature, have deficiencies in their ability to up-regulate neutrophil production and function. Schibler et al. (1993) measured serum G-CSF levels in neutropenic and non-neutropenic neonates and adults to determine whether defective G-CSF production might be responsible and found no elevation of G-CSF in seven neutropenic neonates in contrast with adults with chemotherapy-induced neutropenia. The role of G-CSF in the regulation of neutrophils in neonates was also investigated in a survey of 64 infants ranging in gestational age from 26 to 42 weeks (Gessler et al., 1993). Uninfected preterm neonates had lower neutrophil counts at birth than term infants and G-CSF levels were lower in neonates of earlier gestational age. After birth, preterm infants were less able to mount a postpartum increase in neutrophil counts suggesting that reduced G-CSF production may occur in neonates, especially if preterm. It was suggested that G-CSF may be useful in the treatment of neonatal infections.

F. Myelodysplasia

Studies of serum cytokine levels in patients with myelodysplastic syndromes (MDS) have been performed to provide a basis for possible CSF therapy and to further understand these syndromes (Verhoef et al., 1993; Zwierzina et al., 1992). In 75 patients studied by Verhoef et al. (1993), IL-6 was detectable in 20% of patients, IL-3 in 29%, and G-CSF was elevated in 57%. GM-CSF was not detectable but was reported to be elevated in nine of 34 patients reported by Zwierzina et al. (1992). Whether the CSFs play a role in the pathogenesis of MDS remains unresolved.

G. Cancer and Cancer Therapy

Circulating hematopoietic cytokines may increase in patients with cancer either as a result of an inflammatory response or because of direct

production by tumor. Tumor-associated neutrophilia has been shown to be due to increased G-CSF production by the cancer in a number of cases (Shimasaki et al., 1992). In contrast, G-CSF levels studied in patients with chronic myeloid leukemia using a very sensitive immunoassay were found to be lower than in normal controls. There was an inverse correlation between blood neutrophil counts and the serum G-CSF level, suggesting a negative feedback mechanism between peripheral neutrophils and G-CSF levels (Saitoh and Shibata, 1994).

Although leukemic blast cells bear receptors for CSFs including G-CSF (Begley et al., 1987b; Budel et al., 1989; Motoji et al., 1989), the number of sites, receptor affinities, and dose-response relationships to the CSFs in colony assays have not differed greatly from non-leukemic cells (Vellenga et al., 1987; Park et al., 1989; Budel et al., 1989). There is no convincing evidence that G-CSF functions as an autocrine growth factor in myeloid leukemia, so at present there is little evidence to implicate G-CSF in the pathogenesis of myeloid leukemia. In certain cases of *de novo* AML, defective G-CSF receptor signaling may contribute to leukemogenesis. In a recent report a novel G-CSF receptor isoform was identified. This variant receptor was unable to transduce growth signals and expression was significantly increased in a patient with acute myeloid leukemia (Dong et al., 1995). Although speculative, it is possible that this may have disturbed maturation signaling by the G-CSF receptor and thereby contributed to leukemogenesis.

To evaluate the effects of cytotoxic therapy on marrow stromal cells, G-CSF and GM-CSF production was measured after exposure to high doses of 4-hydroperoxycyclophosphamide and radiation *in vitro*. No impairment was observed, making it unlikely that myeloablative conditioning therapy with these agents reduces production *in vivo* (Laver et al., 1992). In contrast, reduced GM-CSF and IL-1 production by peripheral blood mononuclear cells was observed in some children receiving maintenance therapy for acute lymphocytic leukemia (ALL). The authors proposed that this may contribute to the susceptibility of such patients to overwhelming infection (Ridgway and Borzy, 1993).

In many of the clinical studies reported, G-CSF levels have been measured in the serum. It is important to recognize that hematopoietic growth factors act locally and systemically to control the generation and activities of leukocytes. Since the circulating concentration of these cytokines is only one of many variables which may determine the net biological outcome, there may not be a direct correlation between levels and a clinical endpoint.

VIII. CLINICAL APPLICATIONS OF G-CSF

G-CSF has been applied clinically as a neutrophil growth and differentiation factor to improve cancer therapy and in the treatment of other neutropenic conditions. Applications include administration:

1. After myelotoxic chemotherapy, to accelerate neutrophil recovery;
2. After high dose chemotherapy to allow increased dose intensity;
3. To accelerate bone marrow engraftment after marrow transplantation;
4. To mobilize myeloid progenitor cells for transplantation; and
5. In the treatment of neutropenic states including severe chronic neutropenia, aplastic anemia and myelodysplasia.

A. Standard-Dose Chemotherapy

Early studies with G-CSF were designed to determine whether it could abrogate episodes of neutropenia and febrile neutropenia after chemotherapy at standard doses. Morstyn et al. (1988) and Gabrilove et al. (1988) demonstrated that administration of non-glycosylated G-CSF (filgrastim) after cytotoxic chemotherapy reduced the duration but not the depth of neutropenia in patients with advanced malignancy. This effect was dose-dependent.

Randomized studies confirmed these initial results. Crawford et al. (1991) and Trillet-Lenoir et al. (1993) randomized patients with small cell lung cancer to receive G-CSF or placebo after the first cycle of chemotherapy. The duration of neutropenia and the incidence of febrile neutropenia (temperature ≥38.2°C, neutrophil count $< 1.0\text{x}10^9$/l) and hospital admission were reduced in both studies (Figure 5). Similar results were obtained in patients receiving chemotherapy for high grade non-Hodgkin's lymphoma (NHL), although in this study the duration of hospitalization was not reduced (Pettengell et al., 1992).

Recently, Maher et al. (1994) reported a randomized, double-blinded trial in which G-CSF (filgrastim) or placebo was administered at the onset of febrile neutropenia in patients with cancer who had received chemotherapy. This study was the first to evaluate the utility of filgrastim for the treatment of episodes of post-chemotherapy neutropenic fever, rather than for its prevention. G-CSF accelerated neutrophil recovery and shortened the duration of febrile neutropenia, although by only one

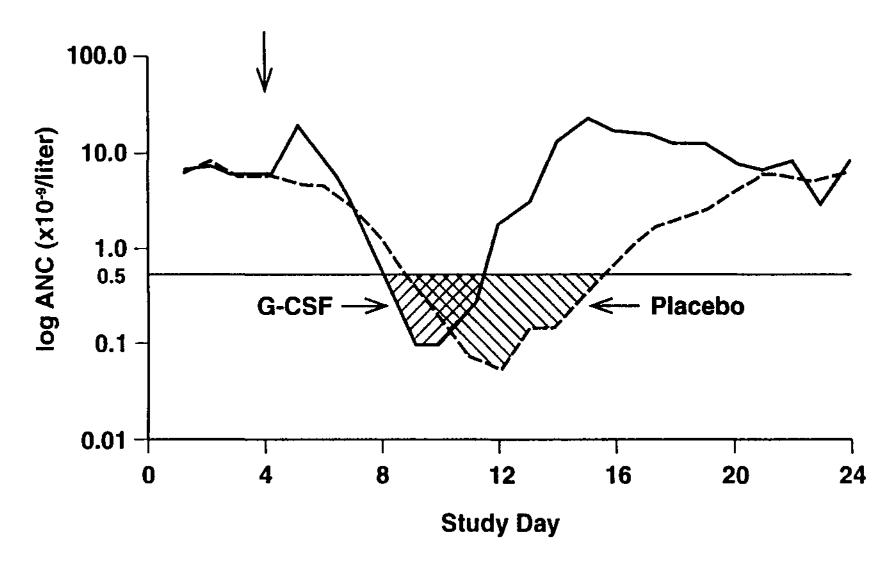


Figure 5. Neutrophil counts as a function of time after myelosuppressive chemotherapy, demonstrating the effect of G-CSF (solid line) compared with a placebo control (broken line). The vertical arrow indicates the day treatment with G-CSF or placebo began (from Crawford et al., 1991).

or two days. The main benefit was in those patients at risk of prolonged neutropenia and in these, hospitalization was significantly reduced.

These studies have all demonstrated an effect on neutrophil counts when G-CSF is administered after chemotherapy. The magnitude of any clinical benefit appears to be greatest when the cytokine is administered prior to the onset of neutropenia. As G-CSF is expensive, its routine use in this manner has obvious cost implications. Pharmacoeconomic analyses suggest that earlier discharge from hospital, reduction in the use of parenteral antibiotics and decreased parenteral nutrition can all lead to overall cost savings (Fox, 1994). However, these findings have recently been disputed (Nichols et al., 1994). The results of prospective assessment of the economic impact of administering G-CSF after chemotherapy are urgently needed.

B. Dose-intensive Chemotherapy

Retrospective analyses describe a relationship between the dose intensity of chemotherapy (defined as mg/m^2/week) delivered and tumor

response in solid tumors and lymphomas (Hrynuik and Bush, 1984; Levin and Hryniuk, 1987; Meyer et al., 1991). Single arm studies of high-dose chemotherapy with autologous progenitor cell rescue suggest that further gains might be attainable with greater escalation in dose (Bosly et al., 1992; Eddy, 1992; Gorin et al., 1993). In the absence of randomized clinical trials, the true benefit of such an approach is yet to be established.

Although G-CSF could prevent delays in the delivery of chemotherapy and maintain planned dose intensity (Trillet-Lenoir et al., 1993; Pettengell et al., 1992), the improvements in delivered dose intensity were modest, and no survival differences were detected in these studies. A large number of phase I and II studies have investigated the ability of growth factors to further increase chemotherapy dose intensity (Basser and Fox, 1995). While they generally demonstrate the feasibility of such an approach, formal assessment demonstrating greater efficacy of these regimens is required.

Clinical application of G-CSF in the treatment of patients with acute leukemia has been slow because G-CSF may also stimulate leukemic cell proliferation (Souza et al., 1986). These fears have been allayed somewhat by clinical studies in patients with relapsed or refractory myeloid leukemia (Ohno et al., 1990) or *de novo* acute myeloid leukemia (Ohno et al., 1993) who received G-CSF following intensive chemotherapy. In these patients, no increase in leukemic relapse was reported. Furthermore, hematological recovery was greatly enhanced, with a consequent reduction in the number of documented infections and days of fever (Ohno et al., 1993).

C. Bone Marrow Transplantation after Myeloablative Chemotherapy

After myeloablative chemotherapy or radiotherapy, hematopoietic stem cell infusion is required to reestablish hematopoiesis. The stem cells can be either autologous or allogeneic. G-CSF has been used after bone marrow transplantation (BMT) in both situations to accelerate neutrophil recovery and enhance the safety of the procedure.

Autologous BMT

Sheridan et al. (1989) found markedly accelerated neutrophil recovery in patients receiving G-CSF 20 μg/kg/day after myeloablative chemo-

therapy and autologous marrow infusion compared with historical controls (time to neutrophils >0.5 x 10^9/l of 11 days vs. 18 days, $p < 0.0005$). This was associated with fewer days of parenteral antibiotics and less days in hospital, but no reduction in neutropenic fever.

Allogeneic BMT

The efficacy of G-CSF is more difficult to evaluate in allogeneic BMT because of the added complexities of graft-versus-host disease (GVHD) and its therapy. In dogs given total body irradiation followed by DLA-identical littermate bone marrow, infusion of G-CSF after transplant led to accelerated white cell recovery, but also a trend toward an increased incidence of GVHD (Schuening et al., 1990). However, phase II (Masaoka et al., 1989) and III (Gisselbrecht et al., 1994) studies have shown no apparent increase in GVHD in patients receiving G-CSF after allogeneic BMT. Neutrophil recovery was greatly enhanced in both studies, and patients given G-CSF had fewer days of infection, antibiotic administration and hospitalization.

D. Peripheral Blood Progenitor Cell Transplantation

Hematopoietic progenitor cells can be mobilized from the marrow into the circulation by administration of G-CSF (Dührsen et al., 1988) or GM-CSF (Socinski et al., 1988; Villeval et al., 1990). This observation has been exploited clinically by supplementing or replacing marrow with peripheral blood progenitor cells (PBPC) collected by leukapheresis during treatment with colony-stimulating factors. In sequential phase II studies, Sheridan et al. (1992) examined the effects on hematopoietic reconstitution of G-CSF-mobilized PBPC (using G-CSF at 12 μg/kg/d for seven days and three aphereses) after myeloablative chemotherapy. Recovery of platelets and neutrophils after bone marrow infusion was accelerated by the addition of PBPC (Sheridan et al., 1992). Subsequently the use of G-CSF-mobilized PBPC alone was found to provide equally rapid reconstitution as long as sufficient numbers of PBPC were reinfused (Sheridan et al., 1994). Only 38% of patients achieved the target number of PBPC (30 x 10^4 CFU-GM/kg) and those with insufficient numbers also required bone marrow after chemotherapy. Increases in the dose or alterations of the schedule of administration of G-CSF did not increase the likelihood of a good yield of PBPC. One method of maximizing yields appears to be with the combination of chemotherapy

and G-CSF (Teshima et al., 1993; Jones et al., 1994). At present there are a number of unresolved issues, including the optimal combination of chemotherapy and growth factor, the dose and schedule of G-CSF, and the timing and number of leukapheresis procedures required.

The need for G-CSF administration to accelerate neutrophil recovery after PBPC infusion is unclear. No randomized data have been published, although data from a phase II study suggest a modest improvement in neutrophil recovery for patients given G-CSF compared with historical controls (Shimazaki et al., 1994).

Early studies have been reported of the use of G-CSF-mobilized PBPC for syngeneic (Weaver et al., 1993) and allogeneic (Dreger et al., 1993; Russell et al., 1993) transplantation. Small numbers of patients have been described, with apparent rapid neutrophil and platelet recovery. Toxicity in the normal donors was minimal. Concerns about more severe acute graft-versus-host disease (GVHD) due to enrichment of T lymphocytes in apheresis collections have been somewhat allayed by these early experiences, however, further studies are required before the overall risk for GVHD can be more completely assessed. A large randomized trial is currently underway in Europe assessing the relative merits of allogeneic PBPC transplant versus BMT.

E. Chronic Neutropenic Syndromes

Congenital neutropenia is usually a severe disorder which presents soon after birth because of infections (Dale and Hammond, 1988). Survival beyond the first few months of life is now possible with appropriate supportive care, although severe infections remain a problem throughout life. There are a number of other congenital neutropenias, including more benign forms (Dale and Vincent, 1994). *Cyclic neutropenia* is characterized by 14 to 28-day fluctuations in blood neutrophils and other white cells, and also reticulocytes and platelets. It is thought to result from a regulatory defect at the level of the stem cell. The main clinical problems are recurrent fever, infections, mucosal ulceration, and lymphadenopathy. *Idiopathic chronic neutropenia* is an acquired disease that occurs in children and adults with no prior history of severe infections and a previously normal blood film (Dale et al., 1979). Most of these patients will maintain a reasonable neutrophil count for prolonged periods, with infrequent infections unless the neutrophils fall below $0.5 \times 10^9/l$.

Many treatments have been tried for these disorders, including splenectomy, endotoxins, corticosteroids, lithium salts, other immunosup-

pressive agents, and gammaglobulins, but they have had little effect on blood neutrophils (Dale and Vincent, 1994). The mainstays of therapy have been careful observation and prompt antibiotic administration.

The availability of G-CSF has provided the first real opportunity to substantially alter the clinical course of patients with severe chronic neutropenic syndromes. Phase I/II reports have demonstrated the ability of G-CSF at doses of 0.6 to 60 μg/kg/d to elevate neutrophil counts in patients with congenital neutropenia (Bonilla et al., 1989; Welte et al., 1990), cyclic neutropenia (Hammond et al., 1989), and chronic idiopathic neutropenia (Jakubowski et al., 1989). This has resulted in marked reduction in the frequency of infection and chronic inflammation. Higher doses of G-CSF are usually required to maintain neutrophil counts at the lower limits of normal in patients with *congenital neutropenia,* where myeloid maturation in the bone marrow is arrested at the promyelocyte stage (Bonilla et al., 1989; Welte et al., 1990). In *cyclic neutropenia,* the oscillations are not abolished by G-CSF, however, the duration of the nadir is dramatically shortened (from six days per cycle to about one day; Hammond et al., 1989). Treatment also shortened the cycle length to about 14 days. Relatively low doses of G-CSF were needed to promptly elevate neutrophils in three patients with *idiopathic neutropenia* (Jakubowski et al., 1989).

A phase III study of 120 patients with severe chronic neutropenic syndromes has confirmed the findings of earlier studies. Administration of G-CSF resulted in elevated neutrophil counts in 108 patients. Mouth ulcers, infections and antibiotic use all decreased significantly (Dale and Vincent, 1994).

Patients in the above trials have been maintained on G-CSF for up to seven years. The clinical benefits of G-CSF have been maintained and the treatment is well tolerated. Reported toxicities have included bone pain early in therapy, mild nonprogressive splenomegaly, hair thinning, rashes, and reduction in platelet counts. A small proportion of patients have developed acute myeloid leukemia, possibly reflecting an underlying pre-malignant condition (Dale et al., 1994). Although G-CSF appears to be safe in most patients, its long-term administration requires careful monitoring.

F. Aplastic Anemia

Aplastic anemia is characterized by a bone marrow that is deficient in hematopoietic progenitor cells (Loughran and Storb, 1990). Colony-

stimulating factors have been used in this disorder in an attempt to enhance production of neutrophils from the small pool of remaining precursor cells. Several phase II studies have demonstrated the activity of G-CSF in moderate or severe aplastic anemia (Kojima et al., 1991; Sonoda et al., 1993). Furthermore, an improvement in anemia and severe thrombocytopenia after 2-10 months of therapy has been observed in some patients (Sonoda et al., 1993).

Recently, Bacigalupo et al. (1993) found raised levels of PBPC in patients with aplastic anemia receiving G-CSF and undergoing leukapheresis. In six of nine treated patients, the total yield of progenitor cells collected (as assessed by granulocyte-macrophage colonies) was in the range described as adequate for PBPC transplantation. This study raises the possibility of using autologous transplantation as a treatment for aplastic anemia.

G. Myelodysplastic Syndromes

Myelodysplastic syndromes (MDS) are a group of disorders characterized by refractory cytopenias and dysplastic changes in blood and bone marrow which carry a high risk of transformation into acute myeloid leukemia. Growth factors offer the potential to reduce rates of infection and transfusion requirements, but also have the theoretical disadvantage of increasing transformation into leukemia. No increase in transformation to acute leukemia has been reported in studies investigating the role of G-CSF in MDS (Negrin et al., 1989, 1990; Kobayashi et al., 1989; Yoshida et al., 1991; Greenberg et al., 1993). Elevation in neutrophil counts of two to 40-fold were achieved, resulting in reduced infection rates.

G-CSF has been combined with erythropoietin to treat MDS after *in vitro* studies showed G-CSF increased the responsiveness of erythroid colonies to erythropoietin in MDS (Stein et al., 1991). A similar phenomenon has now been described in anemic patients with MDS given this combination (Hellstrom-Lindberg et al., 1993; Negrin et al., 1993). Pretreatment erythropoietin levels were lower in responding patients compared with non-responders (Negrin et al., 1993).

The place of G-CSF in the clinic is now well established. However, further data is still required to refine the optimal dose, scheduling, and timing of administration in a number of situations. While cancer therapy and chronic neutropenic states were the obvious early targets for the clinical development of G-CSF, pathophysiology studies suggest it may

also have a role in the treatment of severe, non-neutropenic infections in both the adult and neonate.

IX. CONCLUSIONS

Early studies of G-CSF progressed slowly because of the technical difficulties of purifying a protein that is present at only very low levels in natural sources. The cloning and bacterial expression of G-CSF in 1986 enabled production of large quantities of the protein that were easily purified. Since then, there have been extensive studies of its activities, especially to define its *in vivo* effects and identify clinical applications.

The molecular mechanisms by which G-CSF exerts its effects remain poorly understood. Although its structure has been determined, a detailed understanding of the interaction with its receptor has not yet been achieved and will probably require the determination of the structure of the ligand-receptor complex. Some early events in signal transduction have been identified, but much more work is required before we will fully understand the effects of G-CSF on proliferation and differentiation.

The production of G-CSF "knockout" mice has enabled studies of the normal physiological role of G-CSF in mice and it is to be hoped that identification of further mutations in the receptor in neutropenic patients will increase our knowledge of its importance in man. Another poorly understood effect of G-CSF is its ability to release cells from the bone marrow. This phenomenon is likely to be mediated by changes in cell surface adhesion molecules, but requires further study for a complete understanding.

Several clinical applications for G-CSF have been established and continuing research is likely to identify new therapeutic uses. The use of G-CSF in combination with other cytokines may become important in the future. Although the number of possible combinations (including variations in dose and timing of administration) that may need to be tested seems rather daunting, it may be possible to identify likely combinations in animal studies.

REFERENCES

Abdel-Meguid, S. S., Shieh, H.-S., Smith, W. W., Dayringer, H. E., Violand, B. N., & Bentle, L. A. (1987). Three-dimensional structure of a genetically engineered variant of porcine growth hormone. Proc. Natl. Acad. Sci. USA 84, 6434-6437.

Abramson, J. S., & Hudnor, H. R. (1994). Effect of priming polymorphonuclear leukocytes with cytokines (granulocyte-macrophage colony-stimulating factor [GM-CSF] and G-CSF) on the host resistance to *Streptococcus pneumoniae* in chinchillas infected with influenza A virus. Blood 83, 1929-1934.

Adachi, A., & Saito, H. (1992). Induction of junB expression, but not c-jun, by granulocyte colony-stimulating factor or macrophage colony stimulating factor in the proliferative response of human myeloid leukemia cells. J. Clin. Invest. 89, 1657-1661.

Aloisi, F., Care, A., Borsellino, G., Gallo, P., Rosa, S., Bassani, A., Cabbibo, A., Testa, U., Levi, G., & Peschle, C. (1992). Production of hemolymphopoietic cytokines (IL-6, IL-8, colony-stimulating factors) by normal human astrocytes in response to IL-1β and tumor necrosis factor-α. J. Immunol. 149, 2358-2366.

Asano, M., & Nagata, S. (1992). Constitutive and inducible factors bind to regulatory element 3 in the promotor of the gene encoding mouse granulocyte colony-stimulating factor. Gene 121, 371-375.

Avalos, B.R., Gasson, J.C., Hedvat, C., Quan, S.G., Baldwin, G.C., Weisbart, R.H., Williams, R.E., Golde, D.W., & DiPersio, J.F. (1990). Human granulocyte colony-stimulating factor: biologic activities and receptor characterization on hematopoietic cells and small cell lung cancer cell lines. Blood 75, 851-857.

Bacigalupo, A., Piaggio, G., Podesta, M., Van Lint, M. T., Valbonesi, M., Lercari, G., Mori, P. G., Pasino, M., Franchini, E., Rivabella, L., Figari, O., Sogno, G., Raffo, M. R., & Marmont, A. M. (1993). Collection of peripheral blood hematopoietic progenitors (PBHP) from patients with severe aplastic anemia (SAA) after prolonged administration of granulocyte colony-stimulating factor. Blood 82, 1410-1414.

Baines, P., Mayani, H., Bains, M., Fisher, J., Hoy, T., & Jacobs, A. (1988). Enrichment of CD34 (My10)-positive myeloid and erythroid progenitors from human marrow and their growth in cultures supplemented with recombinant human granulocyte-macrophage colony-stimulating factor. Exp. Hematol. 16, 785-789.

Baiocchi, G., Scambia, G., Benedetti, P., Menichella, G., Testa, U., Pierelli, L., Martucci, R., Foddai, M.L., Bizzi, B., Mancuso, S., & Peschle, C. (1993). Autologous stem cell transplantation: Sequential production of hematopoietic cytokines underlying granulocyte recovery. Cancer Res. 53, 1297-1303.

Baldwin, G. C., Chung, G. Y., Kaslander, C., Esmail, T., Reisfeld, R. A., & Golde, D. W. (1993). Colony-stimulating factor enhancement of myeloid effector cell cytotoxicity towards neuroectodermal tumour cells. Brit. J. Haematol. 83, 545-553.

Bashey, A., Healy, L., & Marshall, C.J. (1994). Proliferative but not nonproliferative responses to granulocyte colony-stimulating factor are associated with rapid activation of the $p21^{ras}$/MAP kinase signalling pathway. Blood 83, 949-957.

Basser, R. L., & Fox, R. M. (1996). Dose-intensive chemotherapy without hemopoietic cell support. In: *Cell Therapy* (Morstyn, G., & Sheridan, W. P., Eds.). Cambridge University Press, pp. 269-298.

Baum, C. M., Weissman, I. L., Tsukamoto, A. S., Buckle, A-M., & Peault, B. (1992). Isolation of a candidate human hematopoietic stem-cell population. Proc. Natl. Acad. Sci. USA 89, 2804-2808.

Bazan, J. F. (1990). Haemopoietic receptors and helical cytokines. Immunol. Today 11, 350-355.

Begley, C. G., Lopez, A. F., Nicola, N. A., Warren, D. J., Vadas, M. A., Sanderson, C. J., & Metcalf, D. (1986). Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils *in vitro*: A rapid and sensitive microassay for colony-stimulating factors. Blood 68, 162-166.

Begley, C. G., Metcalf, D., & Nicola, N. A. (1987a). Purified colony-stimulating factors (G-CSF and GM-CSF) induce differentiation in human HL60 leukemic cells with suppression of clonogenicity. Int. J. Cancer 39, 99-105.

Begley, C.G., Metcalf, D., & Nicola, N.A. (1987b). Primary human myeloid leukemia cells: Comparative responsiveness to proliferative stimulation by GM-CSF or G-CSF and membrane expression of CSF receptors. Leukemia 1, 1-18.

Begley, C. G., Metcalf, D., & Nicola, N. A. (1988a). Binding characteristics and proliferative action of purified granulocyte colony-stimulating factor (G-CSF) on normal and leukemic human promyelocytes. Exp. Hematol. 16, 71-79.

Begley, C. G., Nicola, N. A., & Metcalf D. (1988b). Proliferation of normal human promyelocytes and myelocytes after a single pulse stimulation by purified GM-CSF or G-CSF. Blood 71, 640-645.

Beksac, M., Ozcan, M., Koc, H., Akan, H., Ilhan, O., & Sardas, O.S. (1992). Circulating endogenous granulocyte colony-stimulating factor levels after bone marrow transplantation. Blood 80, 2946-2947.

Bensinger, W. I., Price, T. H., Dale, D. C., Appelbaum, F. R., Clift, R., Lilleby, K., Williams, B., Storb, R., Thomas, E. D., & Buckner, C. D. (1993). The effects of daily recombinant human granulocyte colony-stimulating factor administration on normal granulocyte donors undergoing leukapheresis. Blood 81, 1883-1888.

Bernhardt, T.M., Burchardt, E.R., & Welte, K. (1993). Assessment of G-CSF and GM-CSF mRNA expression in peripheral blood mononuclear cells from patients with severe congenital neutropenia and in human myeloid leukemic cell lines. Exp. Hematol. 21, 163-168.

Bernstein, I. W., Andrews, R. G., & Zsebo, K. M. (1991). Recombinant human stem cell factor enhances the formation of colonies by $CD34^+$ and $CD34^+$ lin^- cells, and the generation of colony forming cell progeny from $CD34^+$ lin^- cells cultured with interleukin-3, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor. Blood 77, 2316-2321.

Bodine, D. M., Crosier, P. S., & Clark, S. C. (1991). Effects of hematopoietic growth factors on the survival of primitive stem cells in liquid suspension culture. Blood 78, 914-920.

Böhmer, R. M., & Burgess, A. W. (1988). Granulocytic colony-stimulating factor (G-CSF) does not induce differentiation of WEHI-3B (D^+) cells but is required for the survival of the mature progeny. Int. J. Cancer 42, 53-58.

Bonilla, M. A., Gillio, A. P., Ruggeiro, M., Kernan, N. A., Brochstein, J. A., Abboud, M., Fumagalli, L., Vincent, M., Gabrilove, J. L., Welte, K., Souza, L. M., & O'Reilly, R. J. (1989). Effects of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with congenital agranulocytosis. N. Engl. J. Med. 320, 1574-1580.

Bosly, A., Coiffier, B., Gisselbrecht, C., Tilly, H., Auzanneau, G., Andrien, F., Herbrecht, R., Legros, M., Devaux, Y., Jaubert, J., Pignon, B., Michaux, J., Humblet, Y., Dupriez, B., Thyss, A., & Lederlin, P. (1992). Bone marrow tranplantation prolongs survival after relapse in aggressive lymphoma patients treated with the LNH-84 regimen. J. Clin. Oncol. 10, 1615-1623.

Brach, M. A., deVos, S., Gruss, H.-J., & Herrmann, F. (1992). Prolongation of survival of human polymorphonuclear neutrophils by granulocyte-macrophage colony-stimulating factor is caused by inhibition of programmed cell death. Blood 80, 2920-2924.

Broudy, V. C., Kaushansky, K., Segal, G. M., Harlan, J. M., & Adamson, J. W. (1986). Tumor necrosis factor type α stimulates human endothelial cells to produce granulocyte/macrophage colony-stimulating factor. Proc. Natl. Acad. Sci. USA 83, 7467-7471.

Broudy, V. C., Kaushansky, K., Harlan, J. M., & Adamson, J. W. (1987). Interleukin-1 stimulates human endothelial cells to produce granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor. J. Immunol. 139, 464-468.

Brown, T. J., Liu, J., Brashem-Stein, C., & Shoyab, M. (1993). Regulation of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor expression by oncostatin M. Blood 82, 33-37.

Buchberg, A. M., Bedigian, H. G., Taylor, B. A., Brownell, E., Ihle, J. N., Nagata, S., Jenkins, N. A., & Copeland, N. G. (1988). Localization of *Evi*-2 to chromosome II: linkage to other proto-oncogene and growth factor loci using interspecific backcross mice. Oncogene Res. 2, 149-165.

Budel, L.M., Touw, I.P., Delwel, R., & Lowenbery, B. (1989). Granulocyte colony-stimulating factor receptors in human acute myelocytic leukemia. Blood 74, 2668-2673.

Burgess, A. W., & Metcalf, D. (1980). Characterization of a serum factor stimulating the differentiation of myelomonocytic leukemic cells. Int. J. Cancer 39, 647-654.

Bussolino, F., Wang, J.M., Defilippi, P., Turrini, F., Sanavio, F., Edgell, C.-J. S., Aglietta, M., Arese, P., & Mantovani, A. (1989). Granulocyte- and granulocyte-macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate. Nature (London) 337, 471-473.

Campbell, I. K., Novak, U., Cebon, J., Layton, J. E., & Hamilton, J. A. (1991). Human articular cartilage and chondrocytes produce hemopoietic colony-stimulating factors in culture in response to IL-1. J. Immunol. 147, 1238-1246.

Casey, L.C., Balk, R.A., & Bone, R.C. (1993). Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. Annals Int. Med. 119, 771-778.

Caspar, C. B., Seger, R. A., Burger, J., & Gmür, J. (1993). Effective stimulation of donors for granulocyte transfusions with recombinant methionyl granulocyte colony-stimulating factor. Blood 81, 2866-2871.

Cebon, J., Layton, J.E., Maher, D., & Morstyn, G. (1994). Endogenous haemopoietic growth factors in neutropenia and infection. Brit. J. Haematol. 86, 265-274.

Chang, J. M., Metcalf, D., Gonda, T. J., & Johnson, G. R. (1989). Long-term exposure to retrovirally expressed granulocyte-colony-stimulating factor induces a non-neoplastic granulocytic and progenitor cell hyperplasia without tissue damage in mice. J. Clin. Invest. 84, 1488-1496.

Chatta, G. S., Price, T. H., Allen, R. C., & Dale, D. C. (1994). Effects of *in vivo* recombinant methionyl human granulocyte colony-stimulating factor on the neutrophil response and peripheral blood colony-forming cells in healthy young and elderly adult volunteers. Blood 84, 2923-2929.

Cheers, C., Haigh, A. M., Kelso, A., Metcalf, D., Stanley, E. R., & Young, A. M. (1988). Production of colony-stimulating factors (CSFs) during infection: Separate determinations of macrophage-, granulocyte-, granulocyte-macrophage- and multi-CSFs. Infect. Immun. 56, 247-251.

Cicuttini, F. M., Martin, M., Salvaris, E., Ashman, L., Begley, C. G., Novotny, J., Maher, D., & Boyd, A. W. (1992). Support of human cord blood progenitor cells on human stromal cell lines transformed by SV_{40} large T antigen under the influence of an inducible (metallothionein) promoter. Blood 80, 102-112.

Clark, S. C., & Kamen, R. (1987). The human hematopoietic colony-stimulating factors. Science 236, 1229-1237.

Cohen, A. M., Zsebo, K. M., Inoue, H., Hines, D., Boone, T. C., Chazin, V. R., Tsai, L., Ritch, T., & Souza, L. M. (1987). *In vivo* stimulation of granulopoiesis by recombinant human granulocyte colony-stimulating factor. Proc. Natl. Acad. Sci. USA 84, 2484-2488.

Corcione, A., Baldi, L., Zupo, S., Dono, M., Rinaldi, G. B., Roncella, S., Taborelli, G., Truini, M., Ferrarini, M., & Pistoia, V. (1994). Spontaneous production of granulocyte colony-stimulating factor *in vitro* by human B-lineage lymphocytes is a distinctive marker of germinal center cells. J. Immunol. 153, 2868-2877.

Corey, S.J., Burkhardt, A. L., Bolen, J. B., Geahlen, R. L., Tkatch, L. S., & Tweardy, D. J. (1994). Granulocyte colony-stimulating factor signaling involves the formation of a three-component complex with Lyn and Syk protein-tyrosine kinases. Proc. Natl. Acad. Sci. USA 91, 4683-4687.

Crawford, J., Ozer, H., Stoller, R., Johnson, D., Lyman, G., Tabbara, I., Kris, M., Grous, J., Picozzi, V., Rausch, G., Smith, R., Gradishar, W., Yahanda, A., Vincent, M., Stewart, M., & Glaspy, J. (1991). Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. N. Engl. J. Med. 325, 164-170.

Cutler, R.L., Liu, L., Damen, J.E., & Krystal, G. (1993). Multiple cytokines induce the tyrosine phosphorylation of Shc and its association with Grb2 in hematopoietic cells. J. Biol. Chem. 268, 21463-21465.

Dale, D. C., & Hammond, W. P. (1988). Cyclic neutropenia: a clinical review. Blood Rev. 2, 178-185.

Dale, D. C., Davis, M., & Vincent, M. E. (1994). Use of Filgrastim (r-metHuG-CSF) in severe chronic neutropenia. In: *Filgrastim (r-metHuG-CSF) in Clinical Practice* (Morstyn, G., & Dexter, T. M., Eds.). Marcel Dekker, Inc., New York, pp. 83-101.

Dale, D. C., Guerry, D., Werwerka, J. R., Bull, J. M., & Chusid, M. H. (1979). Chronic neutropenia. Medicine 58, 128-144.

Dale, D., Bonilla, M. A., Boxer, L., Freedman, M., Brown, S., Fier, C., & Welte, K. (1994). Development of AML/MDS in a subset of patients (pts) with severe chronic neutropenia (SCN). Blood 84, (Suppl 1) 518a (abstr.).

Darnell, J.E., Kerr, I.M., & Stark, G.R. (1994). JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signalling proteins. Science 264, 1415-1421.

de Haas, M., Kerst, J. M., van der Schoot, C. E., Calafat, J., Hack, C. E., Nuijens, J. H., Roos, D., van Oers, R. H. J., & von dem Borne, A. E. G. Kr. (1994). Granulocyte colony-stimulating factor administration to healthy volunteers:

Analysisoftheimmediateactivatingeffectsoncirculatingneutrophils.Blood84, 3885-3894.

de Koning, J.P., Dong, F., Smith, L., Schelen, A.M., Barge, R.M.Y., van der Plas, D.C., Hoefsloot, L.H., Lowenberg, B., & Touw, I.P. (1996). The membrane-distal cytoplasmic region of human granulocyte colony-stimulating factor receptor is required for STAT3 but not STAT1 homodimer formation. Blood 87, 1335-1342.

de Vos, A. M., Ultsch, M., & Kossiakoff, A. A. (1992). Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. Science 255, 306-312.

de Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G., & de Vries, J. E. (1991). Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 174, 1209-1220.

de Wit, H., Dokter, W. H. A., Esselink, M. T., Halie, M. R., & Vellenga, E. (1993). Interferon-γ enhances the LPS-induced G-CSF gene expression in human adherent monocytes, which is regulated at transcriptional and posttranscriptional levels. Exp. Hematol. 21, 785-790.

Demetri, G.D., & Griffin, J.D. (1991). Granulocyte colony-stimulating factor and its receptor. Blood 78, 2791-2808.

Dexter, T. M., Allen, T. D., & Lajtha, L. G. (1977). Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. J. Cell Physiol., 91, 335-344.

Dong, F., van Buttenen, C., Pouwels, K., Hoffesdoot, L.H., Lowenberg, B., & Touw, I.P. (1993). Distinct cytoplasmic regions of the human granulocyte colony-stimulating factor receptor involved in induction of proliferation and maturation. Mol. Cell. Biol. 13, 7774-7781.

Dong, F., Hoefsloot, L. H., Schelen, A. M., Broeders, L. C. A. M., Meijer, Y., Veerman, A. J. P., Touw, I. P., & Löwenberg, B. (1994). Identification of a nonsense mutation in the granulocyte-colony-stimulating factor receptor in severe congenital neutropenia. Proc. Natl. Acad. Sci. USA 91, 4480-4484.

Dong, F., van Paassen, M., van Buitenen, C., Joefsloot, L.H., Löwenberg, B., & Touw, I.P. (1995). A point mutation in the granulocyte colony-stimulating factor receptor (G-CSF-R) gene in a case of acute myeloid leukemia results in the overexpression of a novel G-CSF-R isoform. Blood 85, 902-911.

Dreger, P., Suttorp, M., Haferlach, T., Loffler, M., Schmitz, N., & Schroyerns, W. (1993). Allogeneic granulocyte colony-stimulating factor-mobilized peripheral blood progenitor fro treatment of engraftment failure after bone marrow transplantation. Blood 81, 1404-1407 (letter).

Druker, B.J., Neumann, M., Okuda, K., Franza, B.R., Jr., & Griffin, J. D. (1994). *rel* Is rapidly tyrosine-phosphorylated following granulocyte-colony stimulating factor treatment of human neutrophils. J. Biol. Chem. 269, 5387-5390.

Dubois, C. M., Ruscetti, F. W., Jacobsen, S. E. W., Oppenheim, J. J., & Keller, J. R. (1992). Hematopoietic growth factors upregulate the p65 type II interleukin-1 receptor on bone marrow progenitor cells *in vitro*. Blood 80, 600-608.

Dührsen, U., Villeval, J.-L., Boyd, J., Kannourakis, G., Morstyn, G., & Metcalf, D. (1988). Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. Blood 72, 2074-2081.

Eddy, D. M. (1992). High-dose chemotherapy with autologous bone marrow transplantation for the treatment of metastatic breast cancer J. Clin. Oncol. 10, 657-670 (Published erratum appears in J. Clin. Oncol. (1992) October 10(10), 1655-1658); See comments.

Ema, H., Suda, T., Miura, Y., & Nakauchi, H. (1990). Colony formation of clone-sorted human hematopoietic progenitors. Blood 75, 1941-1946.

Ernst, T. J., Ritchie, A. R., Demetri, G. D., & Griffin, J. D. (1989). Regulation of granulocyte- and monocyte-colony stimulating factor mRNA levels in human blood monocytes is mediated primarily at a post-transcriptional level. J. Biol. Chem. 264, 5700-5703.

Evans, J.P.M., Nire-Sluis, A.R., Hoffbrand, V., & Wickremasinghe, R.G. (1990). Binding of G-CSF, GM-CSF, tumor necrosis factor-α, and γ-interferon to cell surface receptors on human myeloid leukemia cells triggers rapid tyrosine and serine phosphorylation of a 75-Kd protein. Blood 75, 88-95.

Fairbairn, L. J., Cowling, G. J., Reipert, B. M., & Dexter, T. M. (1993). Suppression of apoptosis allows differentiation and development of a multipotent hemopoietic cell line in the absence of added growth factors. Cell 74, 823-832.

Fibbe, W. E., van Damme, J., Billiau, A., Voogt, P. J., Duinkerken, N., Kluck, P. M. C., & Falkenburg, J. H. F. (1986). Interleukin-1 (22-K factor) induces release of granulocyte-macrophage colony-stimulating activity from human mononuclear phagocytes. Blood 68, 1316-1321.

Fibbe, W. E., van Damme, J., Billiau, A., Goselink, H. M., Voogt, P. J., van Eeden, G., Ralph, P., Altrock, B. W., & Falkenburg, J. H. F. (1988a). Interleukin-1 induces human marrow stromal cells in long-term culture to produce granulocyte colony-stimulating factor and macrophage colony-stimulating factor. Blood 71, 430-435.

Fibbe, W. E., van Damme, J., Billiau, A., Duinkerken, N., Lurvink, E., Ralph, P., Altrock, B. W., Kaushansky, K., Willemze, R., & Falkenburg, J. H. F. (1988b). Human fibroblasts produce granulocyte-CSF, macrophage-CSF, and granulocyte-macrophage-CSF following stimulation by interleukin-1 and poly(rI).poly(rC). Blood 72, 860-866.

Fox, R. M. (1994). Colony-stimulating factors: Present status and future potential. Pharmaco. Economics 6 (Suppl. 2), 1-8.

Fuh, G., Cunningham, B.C., Fukunaga, R., Nagata, S., Goeddel, D.V., & Wells, J.A. (1992). Rational design of potent antagonists to the human growth hormone receptor. Science 256, 1677-1680.

Fukunaga, R., Ishizaka-Ikeda, E., & Nagata, S. (1990a). Purification and characterization of the receptor for murine granulocyte colony-stimulating factor. J. Biol. Chem. 265, 14008-14015.

Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y., & Nagata, S. (1990b). Expression cloning of a receptor for murine granulocyte colony-stimulating factor. Cell 61, 341-350.

Fukunaga, R., Seto, Y., Mizushima, S., & Nagata, S. (1990c). Three different mRNAs encoding human granulocyte colony-stimulating factor receptor. Proc. Natl. Acad. Sci. USA 87, 8702-8706.

Fukunaga, R., Ishizaka-Ikeda, E., Pan, C.-X., Seto, Y., & Nagata, S. (1991). Functional domains of the granulocyte colony-stimulating factor receptor. EMBO J. 10, 2855-2865.

Fukunaga, R., Ishizaka-Ikeda, E., & Nagata, S. (1993). Growth and differentiation signals mediated by different regions in the cytoplasmic domain of granulocyte colony-stimulating factor receptor. Cell 74, 1079-1087.

Gabrilove, J. L., Jakubowski, A., Scher, H., Sternberg, C., Wong, G., Grous, J., Yagoda, A., Fain, K., Moore, M. A. S., Clarkson, B., Oettgen, H. F., Alton, K., Welte, K., & Souza, L. (1988). Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium. N. Engl. J. Med. 318, 1414-1422.

Gessler, P., Kirchman, N., Kientsch-Engel, R., Haas, N., Lasch, P., & Kachel, W. (1993). Serum concentrations of granulocyte colony-stimulating factor in healthy term and preterm neonates and in those with various diseases including bacterial infections. Blood 82, 3177-3182.

Gisselbrecht, C., Prentice, H. G., Bacigalupo, A., Biron, P., Milpied, N., Rubie, H., Cunningham, D., Legros, M., Pico, J. L., Linch, D. C., Burnett, A. K., Scarffe, J. H., Siegert, W., & Yver, A. (1994). Placebo-controlled phase III trial of lenograstim in bone-marrow transplantation. Lancet 343, 696-700.

Golde, D.W., & Cline, M.J. (1975). Endotoxin-induced release of colony-stimulating activity in man. Proc. Soc. Exp. Biol. Med. 149, 845-848.

Gorin, N. C., Dicke, K., & Lowenberg, B. (1993). High dose therapy for acute myelocytic leukemia treatment strategy: What is the choice? Ann. Oncol. 4 (Suppl. 1), S59-S80.

Greenberg, P., Taylor, K., Larson, R., Koeffler, P., Negrin, R., Saba, H., Ganser, A., Jakubowski, A., Gabrilove, J., Mufti, G., Cruz, J., Hammond, W., Broudy, V., Langley, G. R., Keating, A., Vardiman, J., Lamborn, K., & Brown, S. (1993). Phase III randomized multicenter trial of G-CSF vs observation for myelodysplastic syndromes (MDS). Blood 82 (Suppl. 1), (#768).

Haas, R., Gericke, G., Witt, B., Cayeux, S., & Hunstein, W. (1993). Increased serum levels of granulocyte colony-stimulating factor after autologous bone marrow or blood stem cell transplantation. Exp. Hematol. 21, 109-113.

Hamilton, J. A., Whitty, G. A., Royston, A. K. M., Cebon, J., & Layton, J. E. (1992a). Interleukin-4 suppresses granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating levels in stimulated human monocytes. Immunology 76, 566-571.

Hamilton, J. A., Piccoli, D. S., Cebon, J., Layton, J. E., Rathanaswani, P., McColl, S. R., & Leizer, T. (1992b). Cytokine regulation of colony-stimulating factor (CSF) production in cultured human synovial fibroblasts. II. Similarities and differences in the control of interleukin-1 induction of granulocyte-macrophage CSF and granulocyte-CSF production. Blood 79, 1413-1419.

Hammond, W. P., Price, T. H., Souza, L. M., & Dale, D. C. (1989). Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. N. Engl. J. Med. 320, 1306-1311.

Hammond, W. P., Csiba, E., Canin, A., Hockman, H., Souza, L. M., Layton, J. E., & Dale, D. C. (1991). Chronic neutropenia. A new canine model induced by human granulocyte colony-stimulating factor. J. Clin. Invest. 87, 704-710.

Haylock, D. N., To, L. B., Dowse, T. L., Juttner, C. A., & Simmons, P. J. (1992). Ex vivo expansion and maturation of peripheral blood $CD34^+$ cells into the myeloid lineage. Blood 80, 1405-1412.

Hellstrom-Lindberg, E., Birgegard, Carlsson, M., Carneskog, J., Dahl, I.-M., Dybedal, I., Grimfors, G., Merk, K., Tangen, J.-M., Winqvist, I., & Ost, A. (1993). A combination of granulocyte colony-stimulating factor and erythropoietin may synergistically improve the anemia in patients with myelodysplastic syndromes. Leuk. Lymphoma. 11, 221-228.

Hill, C. P., Osslund, T. D., & Eisenberg, D. (1993). The structure of granulocyte-colony-stimulating factor and its relationship to other growth factors. Proc. Natl. Acad. Sci. USA 90, 5167-5171.

Hiraoka, O., Anaguchi, H., Yamasaki, K., Fukunaga, R., Nagata, S., & Ota, Y. (1994). Ligand binding domain of granulocyte colony-stimulating factor receptor. J. Biol. Chem. 269, 22412-22419.

Hiraoka, O., Anaguchi, H., Asakura, A., & Ota, Y. (1995). Requirement for the immunoglobulin-like domain of granulocyte colony-stimulating factor receptor in formation of a 2:1 receptor-ligand complex. J. Biol. Chem. 270, 25928-25934.

Hirayama, F., Shih, J-P., Awgulewitsch, A., Warr, G. W., Clark, S. C., & Ogawa, M. (1992). Clonal proliferation of murine lymphohemopoietic progenitors in culture. Proc. Natl. Acad. Sci. USA 89, 5907-5911.

Horan, T., Wen, J., Narhi, L., Parker, V., Garcia, A., Arakawa, T., & Philo, J. (1996). Dimerization of the extracellular domain of granulocyte-colony stimulating factor receptor by ligand binding: A monovalent ligand induces 2:2 complexes. Biochemistry 35, 4886-4896.

Hrynuik, W., & Bush, H. (1984). The importance of dose intensity in chemotherapy of metastatic breast cancer. J. Clin. Oncol. 2, 1281-1288.

Ihle, J.N., Witthuhn, B.A., Quelle, F.W., Yamamoto, K., Thierfelder, W.E., Krieder, B., & Silvennoinen, O. (1994). Signalling by the cytokine receptor superfamily: JAKs and STATs. TIBS 19, 222-227.

Ikebuchi, K., Ihle, J. L., Hirai, Y., Wong, G. G., Clark, S. C., & Ogawa, M. (1988). Synergistic factors for stem cell proliferation: further studies of the target stem cells and the mechanism of stimulation by interleukin-1, interleukin-6, and granulocyte colony-stimulating factor. Blood 72, 2007-2014.

Isfort, R.J., & Ihle, J.N. (1990). Multiple hematopoietic growth factors signal through tyrosine phosphorylation. Growth Factors 2, 213-220.

Jacobsen, F. W., Rusten, L. S., & Jacobsen, S. E. W. (1994). Direct synergistic effects of interleukin-7 on *in vitro* myelopoiesis of human $CD34^+$ bone marrow progenitors. Blood 84, 775-779.

Jakubowski, A. A., Souza, L., Kelly, F., Fain, K., Budman, D., Clarkson, B., Bonilla, M. A., Moore, M. A. S., & Gabrilove, J. (1989). Effects of human granulocyte colony-stimulating factor in a patient with idiopathic neutropenia. N. Engl. J. Med. 320, 38-42.

Jones, H. M., Jones, S. A., Watts, M. J., Khwaja, A., Fielding, A., Goldstone, A. H., & Linch, D. C. (1994). Development of a simplified single-apheresis approach for peripheral-blood progenitor-cell tranplantation in previously treated patients with lymphoma. J. Clin. Oncol. 12, 1693-1702.

Kanda, N., Fukushige, S-I., Murotsu, T., Yoshida, M. C., Tsuchiya, M., Asano, S., Kazior, Y., & Nagata, S. (1987). Human gene coding for granulocyte-colony stimulating factor is assigned to the q21-q22 region of chromosome 17. Somat. Cell. Mol. Genet. 13, 679-684.

Katayama, K., Koizumi, S., Ueno, Y., Ohno, I., Ichihara, T., Horita, S., Miyawaki, T., & Taniguchi, N. (1990). Antagonistic effects of interleukin 6 and G-CSF in the later stage of human granulopoiesis *in vitro*. Exp. Hematol. 18, 390-394.

Kaushansky, K., Lin, N., & Adamson, J. W. (1988). Interleukin 1 stimulates fibroblasts to synthesize granulocyte-macrophage and granulocyte colony-stimulating factors. Mechanism for the hematopoietic response to inflammation. J. Clin. Invest. 81, 92-97.

Kawakami, M., Tsutsumi, H., Kumakawa, T., Abe, H., Hirai, M., Kurosawa, S., Mori, M., & Fukushima, M. (1990). Levels of serum granulocyte colony-stimulating factor in patients with infections. Blood 76, 1962-1964.

Kawano, Y., Takaue, Y., Saito, S., Sato, J., Shimizu, T., Suzue, T., Hirao, A., Okamoto, Y., Abe, T., Watanabe, T., Kuroda, Y., Kimura, F., Motoyoshi, K., & Asano, S. (1993). Granulocyte colony-stimulating factor (CSF), macrophage-CSF, granulocyte-macrophage CSF, interleukin-3, and interleukin-6 levels in sera from children undergoing blood stem cell autografts. Blood 81, 856-860.

Khwaja, A., Carver, J. E., & Linch, D. C. (1992). Interactions of granulocyte-macrophage colony-stimulating factor (CSF), granulocyte CSF, and tumor necrosis factor α in the priming of the neutrophil respiratory burst. Blood 79, 745-753.

Kitagawa, S., Yuo, A., Souza, L. M., Saito, M., Miura, Y., & Takaku, F. (1987). Recombinant human granulocyte colony-stimulating factor enhances superoxide release in human granulocytes stimulated by the chemotactic peptide. Biochem. Biophys. Res. Commun. 144, 1143-1146.

Kittler, E. L. W., McGrath, H., Temeles, D., Crittenden, R. B., Kister, V. K., & Quesenberry, P. J. (1992). Biological significance of constitutive and subliminal growth factor production by bone marrow stroma. Blood 79, 3168-3178.

Kobayashi, Y., Okabe, T., Ozawa, K., Chiba, S., Hino, M., Miyazono, K., Urabe, A., & Takaku, F. (1989). Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor: A preliminary report. Am. J. Med. 86, 178-182.

Koeffler, H. P., Gasson, J., Ranyard, J., Souza, L., Shepard, M., & Munker, R. (1987). Recombinant human TNFα stimulates production of granulocyte colony-stimulating factor. Blood 70, 55-59.

Koeffler, H. P., Gasson, J., & Tobler, A. (1988). Transcriptional and posttranscriptional modulation of myeloid colony-stimulating factor expression by tumor necrosis factor and other agents. Mol. Cell. Biol. 8, 3432-3438.

Koike, K., Ma, F., Shiohara, M., Kubo, T., Takagai, M., Amano, M., Nakahata, T., & Komiyama, A. (1992). Interferon-gamma inhibits proliferation, but not commitment, of murine granulocyte-macrophage progenitors. J. Cell. Physiol. 153, 528-533.

Kojima, S., Fukuda, M., Miyajima, Y., Matsuyama, T., & Horibe, K. (1991). Treatment of aplastic anemia in chidren with recombinant granulocyte colony-stimulating factor. Blood 77, 937-941.

Kubota, N., Orita, T., Hattori, K., Oh-eda, M., Ochi, N., & Yamazaki, T. (1990). Structural characterization of natural and recombinant human granulocyte colony stimulating factors. J. Biochem. (Tokyo) 107, 486-492.

Kuga, T., Komatsu, Y., Yamasaki, M., Sekine, S., Miyaji, H., Nishi, T., Sato, M., Yokoo, Y., Asano, M., Okabe, M., Morimoto, M., & Itoh, S. (1989). Mutagenesis of

human granulocyte colony stimulating factor. Biochem. Biophys. Res. Commun. 159, 103-111.

Lanfrancone, L., Boraschi, D., Ghiari, P., Falini, B., Grignani, F., Peri, G., Mantovani, A.,& Pelicci, P. G. (1992). Human peritoneal mesothelial cells produce many cytokines (Granulocyte colony-stimulating factor [CSF], granulocyte-monocyte-CSF, macrophage-CSF, interleukin-1 [IL-1], and IL-6) and are activated and stimulated to grow by IL-1. Blood 80, 2835-2842.

Lardon, F., Van Bockstaele, D. R., Snoek, H. W., & Peetermans, M. E. (1993). Quantitative cell-cycle progression analysis of the first three successive cell cycles of granulocyte colony-stimulating factor and/or granulocyte-macrophage colony-stimulating factor-stimulated human $CD34^+$ bone marrow cells in relation to their colony formation. Blood 81, 3211-3216.

Larsen, A., Davis, T., Curtis, B.M., Gimpel, S., Sims, J.E., Cosman, D., Park, L., Sorenson, E., March, C.J., & Smith, C.A. (1990). Expression cloning of a human granulocyte colony-stimulating factor receptor: A structural mosaic of hematopoietin receptor, immunoglobulin, and fibronectin domains. J. Exp. Med. 172, 1559-1570.

Laver, J.H., Xu, F., Barredo, J.C., & Abboud, M.R. (1992). Effects of radiation and 4-hydroperoxycyclophosphamide on production of G- and GM-CSF by stromal cells. Bone Marrow Transplant. 10, 529-533.

Layton, J.E., Hockman, H., Sheridan, W.P., & Morstyn, G. (1989). Evidence for a novel in vivo control mechanism of granulopoiesis: Mature cell-related control of a regulatory growth factor. Blood 74, 1303-1307.

Layton, J. E., Morstyn, G., Fabri, L. J., Reid, G. E., Burgess, A. W., Simpson, R. J. and Nice, E. C. (1991). Identification of a functional domain of human granulocyte colony-stimulating factor using neutralizing monoclonal antibodies. J. Biol. Chem. 266, 23815-23823.

Lee, M. Y., Fevold, K. L., Dorshkind, K., Fukunaga, R., Nagata, S., & Rosse, C. (1993). *In vivo* and *in vitro* suppression of primary B lymphocytopoiesis by tumor-derived and recombinant granulocyte colony-stimulating factor. Blood 82, 2062-2068.

Leizer, T., Cebon, J., Layton, J. E., & Hamilton, J. A. (1990). Cytokine regulation of colony-stimulating factor production in cultured human synovial fibroblasts: I. Induction of GM-CSF and G-CSF production by interleukin-1 and tumor necrosis factor. Blood 76, 1989-1996.

Levin, L., & Hryniuk, W. M. (1987). Dose intensity analysis of chemotherapy regimens in ovarian carcinoma. J. Clin. Oncol. 5, 756-767.

Li, J., Koay, D. C., Xiao, H., & Sartorelli, A. C. (1993). Regulation of the differentiation of WEHI-3B D^+ leukemia cells by granulocyte colony-stimulating factor receptor. J. Cell Biol. 120, 1481-1489.

Lieschke, G. J., Grail, D., Hodgson, G., Metcalf, D., Stanley, E., Cheers, C., Fowler, K. J., Basu, S., Zhan, Y. F., & Dunn, A. R. (1994). Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 84, 1737-1746.

Lopez, A. F., Nicola, N. A., Burgess, A. W., Metcalf, D., Battye, F. L., Sewell, W. A., & Vadas, M. (1983). Activation of granulocyte cytotoxic function by purified mouse colony-stimulating factors. J. Immunol. 131, 2983-2988.

Lord, B. I., Bronchud, M. H. Owens, S., Chang, J., Howell, A., Souza, L., & Dexter, T. M. (1989). The kinetics of human granulopoiesis following treatment with granulocyte colony-stimulating factor *in vivo*. Proc. Natl. Acad. Sci. USA 86, 9499-9503.

Lord, B. I., Molineux, G., Pojda, Z., Souza, L. M., Mermod, J-J., & Dexter, T. M. (1991). Myeloid cell kinetics in mice treated with recombinant interleukin-3, granulocyte colony-stimulating factor (CSF), or granulocyte-macrophage CSF *in vivo*. Blood 77, 2154-2159.

Loughran, T. P., Jr., & Storb, R. (1990). Treatment of aplastic anemia. Hematol. Oncol. Clin. North Am. 4, 559-575.

Lovejoy, B., Cascio, D., & Eisenberg, D. (1993). Crystal structure of canine and bovine granulocyte-colony stimulating factor (G-CSF). J. Mol. Biol. 234, 640-653.

Lu, H. S., Boone, T. C., Souza, L. M., & Lai, P-H. (1989). Disulfide and secondary structures of recombinant human granulocyte colony stimulating factor. Arch. Biochem. Biophys. 268, 81-92.

Lu, L., Xiao, M., Shen, R.-N., Grigsby, S., & Broxmeyer, H. E. (1993). Enrichment, characterization, and responsiveness of single primitive $CD34^{+++}$ human umbilical cord blood hematopoietic progenitors with high proliferative and replating potential. Blood 81, 41-48.

Maher, D. W., Lieschke, G. J., Green, M. D., Bishop, J., Stuart-Harris, R., Wolf, M., Sheridan, W. P., Kefford, R. F., Cebon, J., Olver, I., McKendrick, J., Toner, G., Bradstock, K., Lieschke, M., Cruickshank, S., Tomita, D. K., Hoffman, E. W., Fox, R. M., & Morstyn, G. (1994). Filgrastim in patients with chemotherapy-induced febrile neutropenia: A double-blind, placebo-controlled trial. Ann. Int. Med. 121, 492-501.

Malipiero, U. V., Frei, K., & Fontana, A. (1990). Production of hemopoietic colony-stimulating factors by astrocytes. J. Immunol. 144, 3816-3821.

Mangan, K.F., Mullaney, M.T., Diaz Barrientos, T., & Kernan, N.A. (1993). Serum interleukin-3 levels following autologous or allogeneic bone marrow transplantation: Effects of T-cell depletion, blood stem cell infusion, and hematopoietic growth factor treatment. Blood 81, 1915-1922.

Martin, F. H., Suggs, S. V., Langley, K. E., Lu, H. S., Ting, J., Okino, K. H., Morris, C. F., McNiece, I. K., Jacobsen, F. W., Mendiaz, E. A., Birkett, N. C., Smith, K. A., Johnson, M. J., Parker, V. P., Flores, J. C., Patel, A. C., Fischer, E. F., Erjavec, H. O., Herrera, C. J., Wypych, J., Sachdev, R. K., Pope, J. A., Leslie, I., Wen, D., Lin, C-H., Cupples, R. L., & Zsebo, K. M. (1990). Primary structure and functional expression of rat and human stem cell factor DNAs. Cell 63, 203-211.

Masaoka, T., Takaku, F., Kato, S., Moriyama, Y., Kodera, Y., Kanamaru, A., Shimosaka, A., Shibata, H., & Nakamura, H. (1989). Recombinant human granulocyte colony-stimulating factor in allogeneic bone marrow transplantation. Exp. Hematol. 17, 1047-1050.

Mayani, H., Dragowska, W., & Lansdorp, P. M. (1993). Cytokine-induced selective expansion and maturation of erythroid versus myeloid progenitors from purified cord blood precursor cells. Blood 81, 3252-3258.

McNiece, I., Andrews, R., Stewart, M., Clark, S., Boone, T., & Quesenberry, P. (1989). Action of interleukin-3, G-CSF, and GM-CSF on highly enriched human hematopoietic progenitor cells: synergistic interaction of GM-CSF plus G-CSF. Blood 74, 110-114.

McNiece, I. K., Langley, K. E., & Zsebo, K. M. (1991). Recombinant human stem cell factor synergises with GM-CSF, G-CSF, IL-3 and Epo to stimulate human progenitor cells of the myeloid and erythroid lineages. Exp. Hematol. 19, 226-231.

Mempel, K., Pietsch, T., Menzel, T., Zeidler, C., & Welte, K. (1991). Increased serum levels of granulocyte colony-stimulating factor in patients with severe congenital neutropenia. Blood 77, 1919-1922.

Metcalf, D. (1984). The hemopoietic colony stimulating factors. Elsevier, Amsterdam.

Metcalf, D. (1986). The molecular biology and functions of the granulocyte-macrophage colony-stimulating factors: Review. Blood 67, 257-267.

Metcalf, D. (1991). Lineage commitment of hemopoietic progenitor cells in developing blast colonies: influence of colony-stimulating factors. Proc. Natl. Acad. Sci. USA 88, 11310-11314.

Metcalf, D., & Nicola, N. A. (1983). Proliferative effects of purified granulocyte colony-stimulating factor (G-CSF) on normal mouse hemopoietic cells. J. Cell. Physiol. 116, 198-206.

Metcalf, D., & Nicola, N. A. (1992). The clonal proliferation of normal mouse hematopoietic cells: Enhancement and suppression by colony-stimulating factor combinations. Blood 79, 2861-2866.

Meyer, R. M., Hryniuk, W. M., & Goodyear, M. D. E. (1991). The role of dose intensity in determining outcome in intermediate-grade non-Hodgkin's lymphoma. J. Clin. Oncol. 9, 339-347.

Migliaccio, A. R., Migliaccio, G., Adamson, J. W., & Torok-Storb, B. (1992a). Production of granulocyte colony-stimulating factor and granulocyte/macrophage-colony-stimulating factor after interleukin-1 stimulation of marrow stromal cell cultures from normal or aplastic anemia donors. J. Cell. Physiol. 152, 199-206.

Migliaccio, G., Migliaccio, A. R., Druzin, M. L., Giardina, P.-J. V., Zsebo, K. M., & Adamson, J. W. (1992b). Long-term generation of colony-forming cells in liquid culture of $CD34^+$ cord blood cells in the presence of recombinant human stem cell factor. Blood 79, 2620-2627.

Montminy, M. (1993). Trying on a new pair of SH2s. Science 261, 1694-1695.

Morikawa, K., Miyawaki, T., Oseko, F., Morikawa, S., & Imai, K. (1993). G-CSF enhances the immunoglobulin generation rather than the proliferation of human B lymphocytes. Eur. J. Haematol. 51, 144-151.

Morstyn, G., & Burgess, A. W. (1988). Hemopoietic growth factors: A review. Cancer Res. 48, 5624-5637.

Morstyn, G., Campbell, L., Souza, L. M., Alton, N. K., Keech, J., Green, M., Sheridan, W., Metcalf, D., & Fox, R. (1988). Effect of granulocyte colony stimulating factor on neutropenia induced by cytotoxic chemotherapy. Lancet 1, 667-672.

Motoji, T., Takanashi, M., Fuchinoue, M., Masuda, M., Oshimi, K., & Mizoguchi, H. (1989). Effect of recombinant GM-CSF and recombinant G-CSF on colony formation of blast progenitors in acute myeloblastic leukemia. Exp Hematol. 17, 56-60.

Muench, M. O., Schneider, J. G., & Moore, M. A. S. (1992). Interactions among colony-stimulating factors, IL-1β IL-6, and *Kit*-ligand in the regulation of primitive murine hematpoietic cells. Exp. Hematol. 20, 339-349.

Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamazaki, T., Yamamoto, O., Hirata, Y., Kubota, N., Oheda, M., Nomura, H., & Ono, M. (1986a). Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. Nature (London) 319, 415-418.

Nagata, S., Tsuchiya, M., Asano, S., Yamamoto, O., Hirata, Y., Kubota, N., Oheda, M., Nomura, H., & Yamazaki, T. (1986b). The chromosomal gene structure and two mRNAs for human granulocyte colony-stimulating factor. EMBO J. 5, 575-581.

Narazaki, M., Witthuhn, B.A., Yoshida, K., Silvennoinen, O., Yasukawa, K., Ihle, J.N., Kishimoto, T., & Taga, T. (1994). Activation of JAK2 kinase mediated by the interleukin 6 signal transducer gp130. Proc. Natl. Acad. Sci. USA 91, 2285-2289.

Nathan, C. F. (1989). Respiratory burst in adherent human neutrophils: Triggering by colony-stimulating factors CSF-GM and CSF-G. Blood 73, 301-306.

Neben, S., Marcus, K., & Mauch, P. (1993). Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor. Blood 81, 1960-1967.

Negrin, R. S., Haeuber, D. H., Nagler, A., Olds, L. C., Donlon, T., Souza, L. M., & Greenberg, P. L. (1989). Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. A phase I-II trial. Ann. Intern. Med 110, 976-984.

Negrin, R. S., Haeuber, D. H., Nagler, A., Kobayashi, Y., Sklar, J., Donlon, T., Vincent, M., & Greenberg, P. L. (1990). Maintenance treatment of patients with myelodysplastic syndromes using recombinant granulocyte colony-stimulating factor. Blood 76, 36-43.

Negrin, R. S., Doherty, K., Stein, R., Vardiman, J., Krantz, S., & Greenberg, P. L. (1993). Treatment of the anemia of myelodysplastic syndromes using granulocyte colony-stimulating factor in combination with erythropoietin. Blood 82, 737-743.

Nemunaitis, J., Andrews, D. F., Crittenden, C., Kaushansky, K., & Singer, J. W. (1989). Response of simian virus 40 (SV40)- transformed, cultured human marrow stromal cells to hematopoietic growth factors. J. Clin. Invest. 83, 593-601.

Nichols, C. R., Fox, E. P., Roth, B. J., Williams, S. D., Loehrer, P. J., & Einhorn, L. H. (1994). Incidence of neutropenic fever in patients treated with standard-dose combination chemotherapy for small-cell lung cancer and the cost impact of treatment with granulocyte colony-stimulating factor. J. Clin. Oncol. 12, 1245-1250.

Nicholson, S.E., Oates, A.C., Harpur, A.G., Ziemiecki, A., Wilks, A.F., & Layton, J.E. (1994). Tyrosine kinase JAK1 is associated with the granulocyte-colony-stimulating factor receptor and both become tyrosine-phosphorylated after receptor activation. Proc. Natl. Acad. Sci. USA 91, 2985-2988.

Nicholson, S.E., Novak, U., Ziegler, S.F., & Layton, J.E. (1995). Distinct regions of the granulocyte colony-stimulating factor receptor are required for tyrosine phosphorylation of the signaling molecules JAK2, Stat3, and p42, $p44^{MAPK}$. Blood 86, 3698-3704

Nicholson, S.E., Starr, R., Novak, U., Hilton, D.J., & Layton, J.E. (Forthcoming). Tyrosine residues in the granulocyte colony-stimulating factor receptor (G-CSF-R) mediate G-CSF-induced differentiation of mureine myeloid leukemic (M1) cells. J. Biol. Chem.

Nicola, N. (1990). Granulocyte colony-stimulating factor. In: *Colony Stimulating Factors: Molecular and Cellular Biology* (Dexter, T. M., Garland J. M., & Testa N. G., Eds.). Marcel Decker, Inc., New York and Basel, pp. 77-109.

Nicola, N. A., & Metcalf, D. (1984). Binding of the differentiation-inducer, granulocyte-colony-stimulating factor, to responsive but not unresponsive leukemic cell lines. Proc. Natl. Acad. Sci. USA 81, 3765-3769.

Nicola, N.A., & Metcalf, D. (1985). Binding of ^{125}I-labeled granulocyte colony-stimulating factor to normal murine hematopoietic cells. J. Cell. Physiol. 124, 313-321.

Nicola, N. A., & Peterson, L. (1986). Identification of distinct receptors for two hemopoietic growth factors (granulocyte colony-stimulating factor and multipotential colony-stimulating factor) by chemical cross-linking. J. Biol. Chem. 261, 12384-12389.

Nicola, N. A., Metcalf, D., Matsumoto, M., & Johnson, G. R. (1983). Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. J. Biol. Chem. 258, 9017-9023.

Nicola, N.A, Vadas, M.A., & Lopez, A.F. (1986). Down-modulation of receptors for granulocyte colony-stimulating factor on human neutrophils by granulocyte-activating agents. J. Cell. Physiol. 128, 501-509.

Nomura, H., Imazeki, I., Oheda, M., Kubota, N., Tamura, M., Ono, M., Ueyama, Y., & Asano, S. (1986). Purification and characterization of human granulocyte-stimulating factor (G-CSF). EMBO J. 5, 871-876.

Novotny, J. R., Duehrsen, U., Welch, K., Layton, J. E., Cebon, J. S., & Boyd, A. W. (1990). Cloned stromal cell lines derived from human Whitlock/Witte-type long-term bone marrow cultures. Exp. Hematol. 18, 775-784.

O'Neal, K.D., & Yu-Lee, L.-Y. (1993). The proline-rich motif (PRM): A novel feature of the cytokine/hematopoietin receptor superfamily. Lymph. Cyto. Res. 12, 309-312.

Ogawa, M. (1993). Differentiation and proliferation of hematopoietic stem cells. Blood 81, 2844-2853.

Oheda, M., Hasegawa, M., Hattori, K., Kuboniwa, H., Kojima, T., Orita, T., Tomonou, K., Yamazaki, T., & Ochi, N. (1990). O-linked sugar chain of human granulocyte colony-stimulating factor protects it against polymerization and denaturation allowing it to retain its biological activity. J. Biol. Chem. 265, 11432-11435.

Ohno, R., Tomonaga, M., Kobayashi, T., Kanamaru, A., Shirakawa, s., Masaoka, T., Omine, M., Oh, H., Nomura, T., Sakai, Y., Hirano, M., Yokomaku, S., Nakayama, S., Yoshida, Y., Miura, A. B., Morishima, Y., Doht, H., Niho, Y., Hamajima, N., & Takaku, F. (1990). Effect of granulocyte colony-stimulating factor after intensive induction therapy in relapsed or refractory acute leukemia. N. Engl. J. Med. 323, 871-877.

Ohno, R., Hiraoka, A., Tanimoto, A., Asou, N., Kuriyama, K., Kobayashi, T., Yoshida, M., Teshima, H., Saito, H., & Fujimoto, K. (1993). No increase of leukemia relapse in newly diagnosed patients with acute myeloid leukemia who received granulocyte colony-stimulating factor for life-threatening infection during remission induction and consolidation therapy. Blood 81, 561-562 (letter).

Okada, Y., Kawagishi, M., & Kusaka, M. (1990). Effect of recombinant human granulocyte colony-stimulating factor on human neutrophil adherence *in vitro*. Experientia 46, 1050-1053.

Oster, W., Lindemann, A., Mertelsmann, R., & Herrmann, F. (1989). Granulocyte-macrophage colony-stimulating factor (CSF) and multilineage CSF recruit human monocytes to express granulocyte CSF. Blood 73, 64-67.

Pan, C.-X., Fukunaga, R., Yonehara, S., & Nagata, S. (1993). Unidirectional cross-phosphorylation between the granulocyte colony-stimulating factor and interleukin 3 receptors. J. Biol. Chem. 268, 25818-25823.

Papayannopoulou, T., & Nakamoto, B. (1993). Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. Proc. Natl. Acad. Sci. USA 90, 9374-9378.

Park, L. S., Waldron, P. E., Friend, D., Sassenfeld, H. M., Price, V., Anderson, D., Cosman, D., Andrews, R. G., Bernstein, I. D., & Urdal, D. L. (1989). Interleukin-3, GM-CSF, and G-CSF receptor expression on cell lines and primary leukemia cells: Receptor heterogeneity and relationship to growth factor responsiveness. Blood 74, 56-65.

Parry, D. A. D., Minasian, E., & Leach, S.J. (1988). Conformational homologies among cytokines: Interleukins and colony stimulating factors. J. Mol. Recog. 1, 107-110.

Peschel, C., Paul, W. E., Ohara, J., & Green, I. (1987). Effects of B cell stimulatory factor-1/interleukin 4 on hematopoietic progenitor cells. Blood 70, 254-263.

Pettengell, R., Gurney, H., Radford, J. A., Deakin, D. P., James, R., Wilkinson, P. M., Kane, K., Bentley, J., & Crowther, D. (1992). Granulocyte colony-stimulating factor to prevent dose limiting neutropenia in non-Hodgkin's lymphoma: A randomized controlled trial. Blood 80, 1430-1436.

Pojda, Z., Tanaka, K., Aoki, Y., & Tsuboi, A. (1992). *In vivo* and *in vitro* interaction between interleukin 6 and granulocyte colony-stimulating factor in the regulation of murine hematopoiesis. Exp. Hematol. 20, 874-878.

Rabinowitz, J., Petros, W.P., Stuart, A.R., & Peters, W.P. (1993). Characterization of endogenous cytokine concentrations after high-dose chemotherapy with autologous bone marrow support. Blood 81, 2452-2459.

Ravichandran, K.S., & Burakoff, S.J. (1994). The adaptor protein Shc interacts with the interleukin-2 (IL-2) receptor upon IL-2 stimulation. J. Biol. Chem. 269, 1599-1602.

Rennick, D., Yang, G., Gemmell, L., & Lee, F. (1987). Control of hemopoiesis by a bone marrow stromal cell clone: Lipopolysaccharide- and interleukin-1-inducible production of colony stimulating factors. Blood 69, 682-691.

Rennick, D., Jackson, J., Yang, G., Wideman, J., Lee, F., & Hudak, S. (1989). Interleukin-6 interacts with interleukin-4 and other hematopoietic growth factors to selectively enhance the growth of megakaryocytic, erythroid, myeloid, and multipotential progenitor cells. Blood 73, 1828-1835.

Ridgway, D., & Borzy, M.S. (1993). Defective production of granulocyte-macrophage colony-stimulating factor and interleukin-1 by mononuclear cells from children treated for acute lymphoblastic leukemia. Leukemia 6, 809-813.

Roilides, E., Walsh, T.J., Pizzo, P.A., & Rubin, M. (1991). Granulocyte colony-stimulating factor enhances the phagocytic and bactericidal activity of normal and defective human neutrophils. J. Inf. Diseases 163, 579-583.

Russell, N. H., Hunter, A. E., Rogers, S., Hanley, J., & Anderson, D. (1993). Peripheral blood stem cells for allogeneic transplantation. Lancet 341, 1482 (letter).

Saitoh, H., & Shibata, A. (1994). Levels of serum granulocyte colony-stimulating factor in patients with chronic myeloid leukemia. Leuk. Lymphoma 11, 443-446.

Sallerfors, B. (1994). Endogenous production and peripheral blood levels of granulocyte-macrophage (GM-) and granulocyte (G-) colony-stimulating factors. Leukemia Lymphoma 13, 235-247.

Sallerfors, B., & Olofsson, T. (1991). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) in serum during induction treatment of acute leukaemia. Brit. J. Haematol. 78, 343-351.

Sallerfors, B., & Olofsson, T. (1992). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) secretion by adherent monocytes measured by quantitative immunoassays. Eur. J. Haematol. 49, 199-207.

Sato, N., Caux, C., Kitamura, T., Watanabe, Y., Arai, K., Banchereau, J., & Miyajima, A. (1993). Expression and factor-dependent modulation of the interleukin-3 receptor subunits on human hematopoietic cells. Blood 82, 752-761.

Schaafsma, M. R., Falkenburg, J. H. F., Duinkerkin, N., Van Damme, J., Altrock, B. W., Willemze, R., & Fibbe, W. E. (1989). Interleukin-1 synergizes with granulocyte-macrophage colony-stimulating factor on granulocytic colony formation by intermediate production of granulocyte colony-stimulating factor. Blood 74, 2398-2404.

Schibler, K.R., Gechty, K.W., White, W.L., & Christensen, R.D. (1993). Production of granulocyte colony stimulating factor *in vitro* by monocytes from preterm and term neonates. Blood 82, 2478-2484.

Schuening, F. G., Storb, R., Goehle, S., Graham, T. C., Hackman, R., Mori, M., Souza, L. M., & Appelbaum, F. R. (1990). Recombinant human granulocyte colony-stimulating factor accelerates hematopoietic recovery after DLA-identical littermate marrow transplants in dogs. Blood 76, 636-640.

Seelentag, W. K., Mermod, J-J., Montesano, R., & Vassalli P. (1987). Additive effects of interleukin-1 and tumor necrosis factor-α on the accumulation of the three granulocyte and macrophage colony-stimulating factor mRNAs in human endothelial cells. EMBO J. 6, 2261-2265.

Shadduck, R. K., Waheed, A., Greenberger, J. S., & Dexter, T. M. (1983). Production of colony stimulating factor in long-term bone marrow cultures. J. Cell. Physiol. 114, 88-92.

Shannon, M. F., Coles, L. S., Fielke, R. K., Goodall, G. J., Lagnado, C. A., & Vadas, M. A. (1992). Three essential promotor elements mediate tumour necrosis factor and interleukin-1 activation of the granulocyte-colony stimulating factor gene. Growth Factors 7, 181-193.

Sheridan, W. P., Morstyn, G., Wolf, M., Dodds, A., Lusk, J., Maher, D., Layton, J. E., Green, M. D., Souza, L., & Fox, R. M. (1989). Granulocyte colony stimulating factor (G-CSF) and neutrophil recovery following high dose chemotherapy and autologous bone marrow transplantation. Lancet 2, 891-895.

Sheridan, W. P., Begley, C. G., Juttner, C. A., Szer, J., To, L. B., Maher, D., McGrath, K. M., Morstyn, G., & Fox, R. M. (1992). Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. Lancet 339, 640-644.

Sheridan, W. P., Begley, C. G., To, L. B., Grigg, A., Szer, J., Maher, D., Green, M. D., Rowlings, P. A., McGrath, K. M., Cebon, J., Dyson, P., Watson, D., Bayly, J., De Luca, E., Tomita, D., Hoffman, E., Morstyn, G., Juttner, C., & Fox, R. M. (1994). Phase II study of autologous filgrastim-mobilized peripheral blood progenitor cells to restore hemopoiesis after high-dose chemotherapy for lymphoid malignancies. Bone Marrow Trans. 14, 105-111.

Shimane, M., Tani, K., Maruyama, K., Takahashi, S., Ozawa, K., & Asano, S. (1994). Molecular cloning and characterization of G-CSF induced gene cDNA. Biochem. Biophys. Res. Commun. 199, 26-32.

Shimasaki, A. K., Hirata, K., Kawamura, T., Kunibe, N., Hirai, K., Yoshimoto, K., Hashimoto, H., Nakahara, Y., & Mochizuki, Y. (1992). The level of serum granulocyte colony-stimulating factor in cancer patients with leukocytosis. Intern. Med. 31, 861-865.

Shimazaki, C., Oku, N., Uchiyama, H., Yamagata, N., Tatsumi, T., Hirati, T., Ashihara, E., Okawa, K., Goto, H., Inaba, T., Fujita, N., Haruyama, H., & Nakagawa, M. (1994). Effect of granulocyte colony-stimulating factor on hematopoietic recovery after peripheral blood progenitor cell transplantation. Bone Marrow Trans. 13, 271-275.

Shimoda, K., Okamura, S., Harada, N., & Niho, Y. (1992). Detection of the granulocyte colony-stimulating factor receptor using biotinylated granulocyte colony-stimulating factor: Presence of granulocyte colony-stimulating factor receptor on CD34-positive hematopoietic progenitor cells. Res. Exp. Med. 192, 245-255.

Shimoda, K., Okamura, S., Harada, N., Kondo, S., Okamura, T., & Niho, Y. (1993). Identification of a functional receptor for granulocyte colony-stimulating factor on platelets. J. Clin. Invest. 91, 1310-1313.

Shuai, K., Ziemiecki, A., Wilks, A.F., Harpur, A.G., Sadowski, H.B., Gilman, M.Z., & Darnell, J.E. (1993) Polypeptide signalling to the nucleus through tyrosine phosphorylation of JAK and Stat proteins. Nature (London) 366, 580-583.

Simmers, R. N., Webber, L. M., Shannon, M. F., Garson, O. M., Wong, G., Vadas, M. A., & Sutherland, G. R. (1987). Localization of the G-CSF gene on chromosome 17 proximal to the breakpoint in the t (15;17) in acute promyelocytic leukemia. Blood 70, 330-332.

Smith, W. B., Gamble, J. R., & Vadas, M. A. (1994). The role of granulocyte-macrophage and granulocyte colony-stimulating factors in neutrophil transendothelial migration: Comparison with interleukin-8. Exp. Hematol. 22, 329-334.

Snoek, H.-W., Lardon, F., Lenjou, M., Nys, G., Van Bockstaele, D. R., & Peetermans, M. E. (1993). Interferon-γ and interleukin-4 reciprocally regulate the production of monocytes/macrophages and neutrophils through a direct effect on committed monopotential bone marrow progenitor cells. Eur. J. Immunol. 23, 1072-1077.

Socinski, M. A., Cannistra, S. A., Elias, A., Antman, K. H., Schnipper, L., & Griffin, J. D. (1988). Granulocyte-macrophage colony-stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. Lancet 1, 1194-1198.

Sonoda, Y., Okuda, T., Yokota, S., Maekawa, T., Shizumi, Y., Nishigaki, H., Misawa, S., Fujii, H., & Abe, T. (1990). Actions of human interleukin-4/B-cell stimulatory factor-1 on proliferation and differentiation of enriched hematopoietic progenitor cells in culture. Blood 75, 1615-1621.

Sonoda, Y., Ohno, Y., Fujii, H., Takahashi, T., Nakayama, S., Haruyama, H., Nasu, K., Shimazaki, C., Hara, H., Kanamaru, A., Kakishita, E., Horiuchi, A., Abe, T., Masaoka, T., Uchino, H., & Nagai, K. (1993). Multilineage response in aplastic anemia patients following long-term administration of filgrastim (recombinant human granulocyte colony stimulating factor). Stem Cells Dayt. 11, 543-554.

Souza, L. M., Boone, T. C., Gabrilove, J., Lai, P. H., Zsebo, K. M., Murdock, D. C., Chazin, V. R., Bruszewski, J., Lu, H., Chen, K. K., Barendt, J., Platzer, E., Moore, M. A. S., Mertelsman, R., & Welte, K. (1986). Recombinant human granulocyte colony-stimulating factor: Effects on normal and leukemic myeloid cells. Science 232, 61-65.

Spangrude, G. H., Heimfeld, S., & Weissman, I. L., (1988). Purification and characterization of mouse hematopoietic stem cells. Science 241, 58-62.

Stan, A.-C., Walter, G. F., Welte, K., & Pietsch, T. (1994). Immunolocalization of granulocyte-colony-stimulating factor in human glial and primitive neuroectodermal tumors. Int. J. Cancer 57, 306-312.

Stein, R. S., Abels, R. I., & Krantz, S. B. (1991). Pharmacological doses of recombinant erythropoietin in the treatment of myelodysplastic syndromes. Blood 78, 1658-1663.

Sullivan, G. W., Carper, H. T., & Mandell, G. L. (1993). The effect of three human recombinant hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-3) on phagocyte oxidative activity. Blood 81, 1863-1870.

Sutherland, H. J., Hogge, D. E., Cook, D., & Eaves, C. J. (1993) Alternative mechanisms with and without steel factor support primitive human hematopoiesis. Blood 81, 1465-1470.

Taichman, R. S., & Emerson, S. G. (1994). Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J. Exp. Med. 179, 1677-1682.

Tamura, M., Hattori, K., Nomura, H., Oheda, M., Kubota, N., Imazeki, I., Ono, M., Ueyama, Y., Nagata, S., Shirafuji, N., & Asano, S. (1987). Induction of neutrophilic granulocytosis in mice by administration of purified human native granulocyte colony-stimulating factor (G-CSF). Biochem. Biophys. Res. Commun. 142, 454-460.

Temeles, D. S., McGrath, H. E., Kittler, E. L. W., Shadduck, R. K., Kister, V. K., Crittenden, R. B., Turner, B. L., & Quesenberry, P. J. (1993). Cytokine expression from bone marrow derived macrophages. Exp. Hematol. 21, 388-393.

Teshima, T., Harada, M., Takamatsu, Y., Makino, K., Inaba, S., Akashi, K., Kondo, S., Tanaka, T., Ishii, E., & Niho, Y. (1993). Granulocyte colony-stimulating factor (G-CSF)-induced mobilization of circulating haemopoietic stem cells. Br. J Haematol. 84, 570-573.

Thèze, J. (1994). Cytokine receptors: A combinative family of molecules. Eur. Cytokine Netw. 5, 353-368.

Tian, S., Lamb, P., Seidel, H. M., Stein, R. B., & Rosen, J. (1994) Rapid activation of the STAT3 transcription factor by granulocyte colony stimulating factor. Blood 84, 1760-1764.

Torigoe, T., O'Conner, R., Santoli, D., & Reed, J.C. (1992). Interleukin-3 regulates the activity of the Lyn protein-tyrosine kinase in myeloid-committed leukemic cell lines. Blood 80, 617-624.

Trillet-Lenoir, V., Green, J., Manegold, C., Von Pawel, J., Gatzemeler, U., Lebeau, B., Depierre, A., Johnson, P., Decoster, G., Tomita, D., & Ewen, C. (1993). Recombinant granulocyte colony-stimulating factor reduces the infectious complications of cytotoxic chemotherapy. Eur. J. Cancer 29, 319-324.

Tsuchiya, M., Asano, S., Kaziro, Y., & Nagata, S. (1986). Isolation and characterization of the cDNA for murine granulocyte colony-stimulating factor. Proc. Natl. Acad. Sci. USA 83, 7633-7637.

Tsuchiya, M., Kaziro, Y., & Nagata, S. (1987). The chromosomal gene structure for murine granulocyte colony-stimulating factor. Eur. J. Biochem. 165, 7-12.

Tsuji, K., Lyman, S. D., Sudo, T., Clark, S. C., & Ogawa, M. (1992). Enhancement of murine hematopoiesis by synergistic interactions between steel factor (ligand for c-*kit*), interleukin-11, and other early acting factors in culture. Blood 79, 2855-2860.

Tushinski, R. J., Larsen, A., Park, L. S., Spoor, E., Williams, D. E., & Mochizuki, D. Y. (1991). Interleukin 4 alone or in combination with interleukin 1 stimulates 3T3 fibroblasts to produce colony-stimulating factors. Exp. Hematol. 19, 238-244.

Tweardy, D. J., Cannizzaro, L. A., Palumbo, A. P., Shane, S., Heubner, K., Vantuinen, P., Ledbetter, D. H., Finan, J. B., Nowell, P. C., & Rovera, G. (1987). Molecular cloning and characterization of a cDNA for human granulocyte colony-stimulating factor (G-CSF) from a glioblastoma multiforme cell line and localization of the G-CSF gene to chromosome band 17q21. Oncogene Res. 1, 209-220.

Tweardy, D. J., Glazer, E. W., Mott, P. L., & Anderson, K. (1991). Modulation by tumor necrosis factor-α of human astroglial cell production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF). J. Neuroimmunol. 32, 269-278.

Ulich, T. R., del Castillo, J., & Souza, L. (1988). Kinetics and mechanisms of recombinant human granulocyte-colony stimulating factor-induced neutrophilia. Am. J. Pathol. 133, 630-638.

Uzumaki, H., Okabe, T., Sasaki, N., Hagiwara, K., Takaku, F., & Itoh, S. (1988). Characterization of receptor for granulocyte colony-stimulating factor on human circulating neutrophils. Biochem. Biophys. Res. Commun. 156, 1026-1032.

Uzumaki, H., Okabe, T., Sasaki, N., Hagiwara, K., Takaku, F., Tobita, M., Yasukawa, K., Ito, S., & Umezawa, Y. (1989). Identification and characterization of receptors for granulocyte colony-stimulating factor on human placenta and trophoblastic cells. Proc. Natl. Acad. Sci. USA 86, 9323-9326.

Vadas, M. A., Nicola, N. A., & Metcalf, D. (1983). Activation of antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. J. Immunol. 130, 795-799.

Vaillant, P., Muller, V., Martinet, Y., & Martinet, N. (1993). Human granulocyte- and granulocyte-macrophage-colony stimulating factors are chemotactic and "competence" growth factors for human mesenchymal cells. Biochem. Biophys. Res. Commun. 192, 879-885.

Vellenga, E., Young, D.C., Wagner, K., Wiper, D., Ostapovicz, D., & Griffin, J.D. (1987). The effects of GM-CSF and G-CSF in promoting growth of clonogenic cells in acute myeloblastic leukemia. Blood 69, 1771-1776.

Vellenga, E., Rambaldi, A., Ernst, T. J., Ostapovicz, D., & Griffin, J. D. (1988). Independent regulation of M-CSF and G-CSF gene expression in human monocytes. Blood 71, 1529-1532.

Verhoef, G.E.G., De Schouwer, P., Ceuppens, J.L., Van Damme, J., Goossens, W., & Boogaerts, M.A. (1993). Measurement of serum cytokine levels in patients with myelodysplastic syndromes. Leukaemia 6, 1268-1272.

Villeval, J.-L., Dührsen, U., Morstyn, G., & Metcalf, D. (1990). Effect of recombinant human granulocyte-macrophage colony stimulating factor on progenitor cells in patients with advanced malignancies. Br. J. Haematol. 74, 36-44.

Visser, J. W. M., & Van Bekkum, D. W. (1990). Purification of pluripotent hemopoietic stem cells: Past and present. Exp. Hematol. 18, 248-256.

Wang, J. M., Chen, Z. G., Colella, S., Bonilla, M. A., Welte, K., Bordignon, C., & Mantovani, A. (1988). Chemotactic activity of recombinant human granulocyte colony-stimulating factor. Blood 72, 1456-1460.

Watari, K., Asano, S., Shirafuji, N., Kodo, H., Ozawa, K., Takaku, F., & Kamachi, S. (1989). Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood 73, 117-122.

Weaver, C. H., Buckner, C. D., Longin, K., Appelbaum, F. R., Rowley, S., Lilleby, K., Miser, J., Storb, R., Hansen, J. A., & Besinger, W. (1993). Syngeneic transplantation with peripheral blood mononuclear cells collected after the administration of recombinant human granulocyte colony-stimulating factor. Blood 82, 1981-1984.

Welham, M. J., Duronio, V., Leslie, K. B., Bowtell, D., & Schrader, J. W. (1994). Multiple hemopoietins, with the exception of interleukin-4, induce modification of Shc and mSos1, but not their translocation. J. Biol. Chem. 269, 21165-21176.

Welte, K., Platzer, E., Lu, L, Gabrilove, J. L., Levi, E., Mertelsmann, R., & Moore, M. A. S. (1985). Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor. Proc. Natl. Acad. Sci. USA 82, 1526-1530.

Welte, K., Bonilla, M. A., Gillio, A. P., Boone, T. C., Potter, G. K., Gabrilove, J. L., Moore, M. A. S., O'Reilly, R. J., & Souza, L. M. (1987). Recombinant human granulocyte colony-stimulating factor. Effects on hematopoiesis in normal and cyclophosphamide-treated primates. J. Exp. Med. 165, 941-948.

Welte, K., Zeidler, C., Reiter, A., M l ler, W., Odenwald, E., Souza, L., & Riehm, H. (1990). Differential effects of granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor in children with severe congenital neutropenia. Blood 75, 1056-1063.

Werner, J. M., Breeze, A. L., Kara, B., Rosenbrock, G., Boyd, J., Soffe, N., & Campbell, I. D. (1994). Secondary structure and backbone dynamics of human granulocyte colony-stimulating factor in solution. Biochem. 33, 7184-7192.

Wilks, A.F., & Harpur, A.G. (1994). Cytokine signal transduction and the JAK family of protein tyrosine kinases. Bioessays 16, 313-320.

Williams, G. T., Smith, C. A., Spooncer, E., Dexter, T. M., & Taylor, D. R. (1990). Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. Nature (London) 343, 76-79.

Witthuhn, B.A., Quelle, F.W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., & Ihle, J.N. (1993). JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 74, 227-236.

Yamamoto, Y., Klein, T. W., Friedman, H., Kimura, S., & Yamaguchi, H. (1993). Granulocyte colony-stimulating factor potentiates *anti-Candida albicans* growth inhibitory activity of polymorphonuclear cells. FEMS Immunol. Med. Microbiol. 7, 15-22.

Yang, Y-C., Tsai, S., Wong, G. G., & Clark, S. C. (1988). Interleukin-1 regulation of hematopoietic growth factor production by human stromal fibroblasts. J. Cell. Physiol. 134, 292-296.

Yong, L. L., & Linch, D. C. (1992). Differential effects of granulocyte- and granulocyte-macrophage colony-stimulating factors (G- and GM-CSF) on neutrophil adhesion *in vitro* and *in vivo*. Eur. J. Haematol. 49, 251-259.

Yong, K., Cohen, H., Khwaja, A., Jones, H. M., & Linch, D. C. (1991). Lack of effect of granulocyte-macrophage and granulocyte colony-stimulating factors on cultured human endothelial cells. Blood 77, 1675-1680.

Yoshida, Y., Hirashima, K., Asano, S., & Takaku, F. (1991). A phase II trial of recombinant human granulocyte colony-stimulating factor on the myelodysplastic syndromes. Br. J. Haematol. 78, 378-384.

Yoshikubo, T., Ozawa, K., Takahashi, K., Nishikawa, M., Horiuchi, N., Tojo, A., Tani, K., Kodama, H., & Asano, S. (1994). Adhesion of NFS-60 myeloid leukemia cells to MC3T3-G2/PA6 stromal cells induces granulocyte colony-stimulating factor production. Blood 84, 415-420.

Yuo, A., Kitagawa, S., Ohsaka, A., Saito, M., & Takaku, F. (1990). Stimulation and priming of human neutrophils by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor: Qualitative and quantitative differences. Biochem. Biophys. Res. Commun. 171, 491-497.

Ziegler, S.F., Bird, T.A., Morella, K.K., Mosley, B., Gearing, D.P., & Baumann, H. (1993). Distinct regions of the human granulocyte-colony stimulating factor receptor cytoplasmic domain are required for proliferation and gene induction. Mol. Cell. Biol. 13, 2384-2390.

Zink, T., Ross, A., Luers, K., Cieslar, C., Rudolph, R., & Holak, T. A. (1994). Structure and dynamics of the human granulocyte colony-stimulating factor determined by NMR spectroscopy. Loop mobility in a four-helix-bundle protein. Biochem. 33, 8453-8463.

Zoellner, H., Filonzi, E. L., Stanton, H. R., Layton, J. E., & Hamilton, J. A. (1992). Human arterial smooth muscle cells synthesize granulocyte colony-stimulating factor in response to interleukin-1α and tumor necrosis factor-α. Blood 80, 2805-2810.

Zoellner, H., Cebon, J., Layton, J. E., Stanton, H., & Hamilton, J. A. (1993). Contrasting effects of interleukin-4 on colony-stimulating factor and interleukin-6 synthesis by vascular endothelial cells. Lymphokine Cytokine Res. 12, 93-99.

Zsebo, K. M., Cohen, A. M., Murdock, D. C., Boone, T. C., Inoue, H., Chazin, V. R., Hines, D., & Souza, L. M. (1986). Recombinant human granulocyte colony stimulating factor: Molecular and biological characterization. Immunobiol. 172, 175-184.

Zsebo, K. M., Yuschenkoff, V. N., Schiffer, S., Chang, D., McCall, E., Dinarello, C. A., Brown, M. A., Altrock, B., & Bagby, G. C., Jr. (1988). Vascular endothelial cells and granulopoiesis: Interleukin-1 stimulates release of G-CSF and GM-CSF. Blood 71, 99-103.

Zwierzina, H., Schollenberger, S., Herold, M., Schmalzl, F., & Besemer, J. (1992). Endogenous serum levels and surface receptor expression of GM-CSF and IL-3 in patients with myelodysplastic syndromes. Leukemia Res. 16, 1181-1186.

LEUKEMIA INHIBITORY FACTOR AND ITS RECEPTOR

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I. PROPERTIES AND PRODUCTION OF LIF

A. LIF Protein

The initial purification of murine and human LIF revealed that they were heavily N-glycosylated proteins of Mr 32 to 58,000 with a high isoelectric point of 8.5 to greater than 9.5 (Godard et al., 1988; Hilton et al., 1988; Tomida et al., 1984). LIF appeared to be an extremely stable molecule and retained activity after treatment with strong denaturants (6 M guanidine hydrochloride, sodium dodecyl sulfate), extremes of pH (2-10), and temperature so long as the disulfide bonds remained intact. LIF was sensitive, however, to strong oxidizing conditions and conditions that allowed modification of free amino groups (Hilton, Layton, and Nicola, unpublished observations).

Molecular cloning of cDNAs encoding murine and human LIF revealed that they encode a protein with a predicted length of 203 amino acids that includes a 23 amino acid leader sequence that is cleaved during secretion (Gearing et al., 1987; Gough et al., 1988). LIF contains seven potential N-glycosylation sites and six cysteines that are conserved in nearly all species examined. The cysteines form three disulfide bonds (Cys 13-135, 19-132 and 61-164) and two of these are conserved in oncostatin-M (Cys 6-127, 49-167) a protein with limited sequence homology to LIF (Nicola et al., 1993; Rose and Bruce, 1991; Figure 1).

Structural predictions based on the amino acid sequence of LIF had suggested that it would have a conformation similar to the long-chain 4-α-helical cytokines, growth hormone (GH), and granulocyte colony-stimulating factor (G-CSF; Bazan, 1991; Nicola et al., 1993). The recent crystallisation and X-ray structure determination of murine LIF (Robinson et al., 1994) has confirmed this prediction.

Murine LIF contains four long helices labeled A-D in an up-up-down-down 4-α-helical bundle topology connected by two long overhand loops A-B and C-D and a short B-C loop. The A-B loop also contains a short helical segment A'. The B and C helices are relatively straight and form an antiparallel helical pair but the A and D helices (especially the A-helix) have pronounced kinks. The N-terminus (residues 1-8) has a

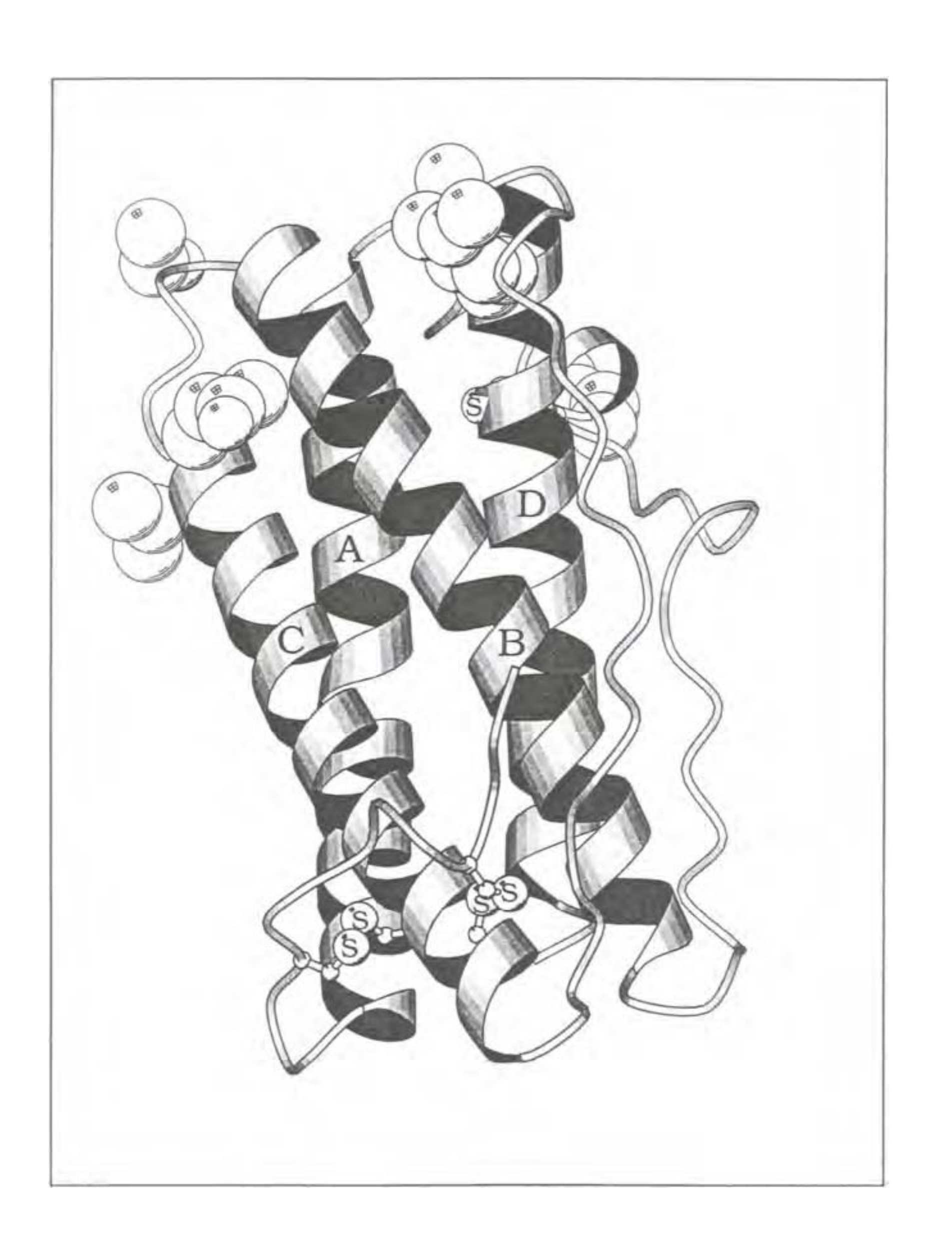


Figure 1. Structure of murine LIF delineated by X-ray crystallography. The main helical segments are labelled A-D from the N-terminus. Residues responsible for binding to the human LIF receptor α-chain are shown in CPK mode and the three disulphide bonds are shown in ball and stick mode (see Robinson et al., 1995).

relatively disordered structure that wraps around the base of the 4-α-helical bundle before the A-helix begins and is tethered to the base of the C-helix by two disulfide bonds. The third disulfide bond (Cys 61-164) links the first part of the A-B loop to the top of the D-helix (Figure 1). These structural features create a compact molecule of dimensions 22 x 28 x 46 A° with a conventional hydrophobic core (Robinson et al., 1994).

LIF exhibits an unusual one-way species cross-reactivity. While mouse and human LIF bind to the mouse LIF receptor α-chain, only human LIF binds to the human LIF receptor chain. Using murine/human LIF chimeras advantage was taken of this observation to map the amino acid residues that define the human LIF receptor binding site (Layton et al., 1992, 1994a; Owczarek et al., 1993). These studies defined six amino acids in human LIF (Asp 57, Ser 107, His 112, Ser 113, Val 155, and Lys 158) which make a significant energetic contribution to human LIF binding to its receptor α-chain (Layton et al., 1994b; Owczarek et al., 1993). Although a larger number of residues usually make receptor contacts in ligand/receptor complexes these data are consistent with similar studies on growth hormone which suggest that only 6-7 of these contacts contribute to the energy of the interaction (Cunningham et al., 1991; de Vos et al., 1992). The six identified residues in human LIF, when placed on the structure determined for mouse LIF (Robinson et al., 1994), form an interaction surface at the top of the molecule (Figure 1; Layton et al., 1994b; Owczarek et al., 1993) and include residues in the A-B loop (A' helix) (Asp 57), the B-C loop (Ser 107), the top of the C-helix (His 112, Ser 113), and the top of the D-helix (Val 155 and Lys 158). This interaction surface is at a quite different position to that determined for the site I receptor interaction of growth hormone (Cunningham et al., 1991; de Vos et al., 1992) despite their similarity in overall structure and their use of structurally related receptor chains.

During the analysis of LIF chimeras it was noted that human LIF bound to the mouse LIF receptor α-chain with a 100-fold higher affinity than did mouse LIF. Surprisingly, the human LIF residues contributing to this behavior were identical with those defining the human LIF receptor binding site (Layton et al., 1994a, 1994b, 1994c; Owczarek et al., 1993). This led to the suggestion that the primary interaction site on LIF for the LIF receptor α-chain was different between mice and humans and that human LIF had evolved to recognize its receptor at a new site while retaining the residues that allow mouse LIF to interact with its receptor α-chain.

The chimeric studies did not address the issue of the interaction site on LIF for the affinity converting β-chain, gp130. However, other

mutagenesis studies by Robinson and colleagues, coupled with structural homologies to the growth hormone/receptor complex, have led them to speculate that LIF might interact with its α-chain at two sites (sites I and III) and with gp130 at a third site (site III; Robinson et al., 1994). Further studies will be required to define the details of the gp130 interaction site.

B. Production of LIF

Under normal circumstances LIF is undetectable in the circulation. Levels are elevated in patients with infection, markedly so in those with sepsis (Kreisberg et al., 1993; Lecron et al., 1993; Waring et al., 1992, 1993; Wesselingh et al., 1994). A similar increase in LIF levels is observed in animals carrying infections or treated with agents such as bacterial lipopolysaccharide (LPS). Surprisingly, treatment of infected animals with LIF appears to alleviate endotoxaemia rather than aggravating it (Alexander et al., 1992; Waring et al., 1995). LIF is also found at high levels in the synovial fluid of patients with rheumatoid arthritis, and has been suggested to play a role in the pathogenesis of this disorder (Campbell et al., 1993; Carroll and Bell, 1993; Hamilton et al., 1993; Ishimi et al., 1992; Lotz et al., 1992; Waring et al., 1993). LIF mRNA is present at elevated levels at the site of nerve and muscle damage, and is believed to be produced by both neuronal and non-neuronal cells (Banner and Patterson, 1994; Barnard et al., 1994; Curtis et al., 1994). As described in the following discussion, LIF promotes the survival and regeneration of damaged nerve and muscle cells.

In vitro correlates of *in vivo* LIF production may also be found. The LIF gene is transcribed and LIF is produced at a low constitutive level by a variety of cells and tissues. Stimulation with agents such as LPS, inflammatory cytokines and T cell activators results in elevated expression of LIF (Aloisi et al., 1994; Anegon et al., 1990; Brown et al., 1994; Derigs and Boswell, 1993; Gascan and Lemetayer, 1991; Greenfield et al., 1993; Mezzasoma et al., 1993; Wetzler et al., 1991). For example, increased production of LIF by chondrocytes and synoviocytes is induced *in vitro* by treatment with LPS, IL-6 and IL-1 (Campbell et al., 1993; Lotz et al., 1992). Likewise astrocytes increase LIF production in response to LPS (Aloisi et al., 1994).

The most dramatic regulation of LIF production is observed in the uterus during the estrous cycle and during pregnancy (Bhatt et al., 1991; Croy et al., 1991; Kojima et al., 1994; Stewart et al., 1992; Yang et al.,

1994). Transcription of the LIF gene is increased again at the time the blastocyst is formed, prior to its implantation in the uterine wall. LIF is detectable in the lumen of the uterus at this time and the site of production of LIF within the uterus is the endometrial glands (Bhatt et al., 1991; Croy et al., 1991). Pseudopregnant mice also exhibit increased expression of LIF suggesting that transcription of the LIF gene is under maternal control and is not affected by the presence of the embryo (Bhatt et al., 1991). Moreover, in situations where blastocyst implantation is delayed, because of the presence of suckling pups or due to ovariectomy and progesterone treatment, LIF production was delayed until the pups were removed or β-oestradiol was administered. LIF expression, as described in the following section, is essential to blastocyst implantation (Stewart et al., 1992).

C. The LIF mRNA and Gene

cDNAs encoding murine, human, rat, ovine, bovine, and porcine LIF have been cloned and are predicted to yield proteins with a high level of amino acid sequence similarity (Table 1; Willson et al., 1992). A lower degree of sequence similarity is observed to CNTF, OSM, and cardiotrophin (CCT1), and to a lesser extent to IL-6, IL-11, and G-CSF (Table 1; Bazan, 1990a, 1990b, 1991; Lin et al., 1989; Pennica et al., 1995; Rose and Bruce, 1991). The genes for LIF lie on syntenic regions of the mouse and human genome—chromosome 22q12 in the human and 11A1 in the

Table 1. Sequence Identity Between Various Species LIF and Related Cytokines

	hLIF	*mLIF*	*rLIF*	*oLIF*	*pLIF*	*OSM*	*CNTF*	*mCT1*	*IL-6*	*G-CSF*
hLIF	—	79	81	88	87	18	16	24	9	11
mLIF		—	92	74	78					
rLIF			—	75	78					
oLIF				—	84					
pLIF					—					
OSM						—	15		8	13
CNTF							—	19	6	12
CT1								—		
IL-6									—	17
G-CSF										—

Source: References for the figures are as follows: the values for the different species of LIF (Willson et al., 1992); those involving OSM, CNTF, IL-6, and G-CSF (Bazan, 1991); and those involving CT1 (Pennica et al., 1995).

Notes: The values given are for the % aminoacid identity found upon pairwise alignment of the various protein sequences. h, human; m, murine; r, rat; o, ovine; p, porcine.

mouse (Kola et al., 1990; Sutherland et al., 1989). In each species examined the LIF mRNA is approximately 4.5 kb long and is transcribed from a gene with three exons and two introns (Stahl et al., 1990). In the human gene, for example, the first exon encodes the 5'-untranslated region and the first six amino acids of the leader sequence, the second exon encodes the remainder of the leader sequence and the first 44 residues of the mature protein, while the third exon encodes the C-terminal 136 residues and an extensive 3'-untranslated region. In the mouse, but apparently not in any other species, an alternative first exon has been described which lies within the usual first intron (Rathjen et al., 1990). Use of this exon requires an unprecedented splicing event (Gough et al., 1992) and is proposed to yield a protein that is identical to previously described LIF species except for the first four residues of the leader sequence (Rathjen et al., 1990). Although the leader sequence is presumably cleaved co-translationally, these differences are claimed to result in the targeting of this alternative to the extracellular matrix rather than allowing it to remain free in the extracellular milieu (Rathjen et al., 1990).

Four TATA elements are found in the 5' region of the murine and human LIF genes (Stahl et al., 1990). The transcriptional start site of murine LIF has been mapped using S1 nuclease protection to a region adjacent to the most proximal TATA element (Stahl et al., 1990). A second start site has also been identified near the second most distal TATA element (Stahl et al., 1990). The minimal LIF promoter has been mapped to nucleotides -103 to 1 by monitoring the ability to drive expression of chloramphenicol acetyl transferase (CAT) expression in STO cells (Stahl and Gough, 1993). A negative regulatory element (-36 to -249) in the murine gene was also identified, however, this sequence was not well conserved in the human gene (Stahl et al., 1990; Stahl and Gough, 1993). A putative enhancer within the murine LIF gene has also been described as having potential steroid responsive elements (Hsu and Heath, 1994; Stahl and Gough, 1993). The latter may be of importance in regulated expression of LIF during pregnancy.

II. BIOLOGICAL EFFECTS OF LIF

A. Overview

The biological effects of LIF have been explored in three ways: (1) by adding LIF to cells growing *in vitro*, (2) by artificially elevating LIF

Table 2. Biological Actions of LIF and Related Cytokines

	LIF	*OSM*	*IL-6*	*IL-11*	*CNTF*
Induction of M1 differentiation.	+1	+2	+3	-4	?
Inhibition of ES cell differentiation.	+5	+6	-7	?	+8
Stimulation of megakaryocyte and platelet formation.	+9	?	+10	+11	?
Stimulation of Kaposi's sarcoma growth.	?	+12	+13	?	
Alteration of neurotransmitter phenotype.	+14	+15	+16	?	+17
Stimulation of neuronal survival.	+18	?	+19	?	+20
Inhibition of lipoprotein lipase.	+21	+22	+23	+24	-25
Stimulation of osteoblast and/or osteoclast growth and function.	+26	+27	+28	+29	?
Stimulation of myoblast growth.	+30	+31	+32	?	+33
Synchronization of blast-CFC proliferation.	+34	?	+35	+36	?
Stimulation of acute phase protein synthesis.	+37	+38	+39	+40	+41
Stimulation of plasmacytoma proliferation.	+42	+43	+44	+45	?

Sources: (1) Metcalf et al., 1988. (2) Bruce et al., 1992. (3) Lotwem et al., 1989. (4) Hilton et al., 1994. (5) Williams et al., 1988. (6) Rose et al., 1994. (7) Yoshida et al., 1994. (8) Conover et al., 1993. (9) Metcalf et al., 1991. (10 Warren et al., 1989. (11) Burstein et al., 1992; Yonemura et al., 1992. (12) Miles et al., 1992; Nair et al., 1992. (13) Miles et al., 1990. (14) Yamamori et al., 1989. (15) Rao et al., 1992. (16) Oh and O'Malley, 1994. (17) Stockli et al., 1989. (18) Martinou et al., 1992; Richards et al., 1992. (19) Kushima and Hatanaka, 1992. (20) Stockli et al., 1989. (21) Mori et al., 1989. (22) Gimble et al., 1994. (23) Greenberg et al., 1992. (24) Yin et al., 1992. (25) Gimbleet al., 1994. (26) Allan et al., 1990. (27) Gimbleet al., 1994. (28) Ishimi et al., 1990. (29) Hughes and Howells, 1993. (30) Austin and Burgess, 1991. (31) Grove et al., 1993. (32) Austin and Burgess, 1991. (33) Helgren et al., 1994. (34) Leary et al., 1991. (35) Ikebuchi et al., 1988. (36) Musashi et al., 1991. (37) Baumann and Wong, 1989. (38) Richards et al., 1993. (39) Gauldie et al., 1987. (40) Baumann and Schendel, 1991. (41) Schooltink et al., 1992. (42) Nishimoto et al., 1994. (43) Nishimoto et al., 1994; Barton et al., 1994. (44) Kawano et al., 1988. (45) Paul et al., 1990.

levels *in vivo* and (3) by preventing the production of LIF *in vivo*. These three approaches have proven complementary and have led to the notion that LIF is an extremely pleiotropic molecule but that many of its actions are shared with other regulators (Table 2). In the following discussion, in order to present a unified picture, we have attempted to integrate information derived from *in vitro* and *in vivo* studies of LIF's effects on its various targets.

B. Haemopoietic Cells

Induction of Myeloid Differentiation in Leukaemic Cell Lines

In retrospect, the first system used to characterize LIF was the murine myeloid leukaemic cell line, M1. The M1 cell line was derived by

Ichikawa from a spontaneous tumor in SL mice (Ichikawa, 1969). In the presence of serum from mice injected with lipopolysaccharide, M1 cells were shown to differentiate into macrophages and to a lesser extent neutrophilic granulocytes (Ichikawa, 1969, 1970). Other stimuli were also found to contain such an activity, which was termed variously, differentiation-inducing factor (D-Factor, DIF; Lowe et al., 1989; Tomida et al., 1984; Yamamoto et al., 1980, 1981), macrophage-granulocyte inducer type 2 (MGI-2A and MGI-2B; Lipton and Sachs, 1981), and later leukemia inhibitory factor (LIF; Gearing et al., 1987; Gough et al., 1988; Hilton et al., 1988a, 1988b). D-Factor, LIF and probably MGI-2B were shown to be identical (Hilton et al., 1988a; 1988b; Lowe et al., 1989), while MGI-2A was found to be equivalent to IL-6 (Shabo et al., 1988). In addition to LIF and IL-6, OSM and to a lesser extent G-CSF have also been shown to induce the macrophage differentiation of M1 cells (Bruce et al., 1992; Lotem et al., 1989; Metcalf, 1989; Tomida et al., 1986; Yamamoto-Yamaguchi et al., 1989).

Stimulation of M1 cells by LIF results in a reduction of myc, myb, and scl/tal-1 mRNA levels and an increase in the level of fos mRNA (Liebermann and Hoffman-Liebermann, 1989; Tanigawa et al., 1993). A systematic study, investigating the nature of the immediate-early response of M1 cells to LIF and IL-6, has found that the levels of other mRNAs also increase. Among these are the mRNAs encoding jun B, c-jun, histones H1 and H3, ICAM, and previously uncharacterized species MyD88 and MyD116 (Lord et al., 1990a, 1990b, 1990c, 1990d). The importance of the decrease in myc, myb, and scl mRNA levels was highlighted by experiments in which M1 cells were engineered to express these proteins constitutively and shown to be blocked in their ability to differentiate in response to LIF and IL-6 (Hoffman-Liebermann and Liebermann, 1991a, 1991b; Selvakumaran et al., 1992; Tanigawa et al., 1993). In contrast, M1 cells constitutively expressing fos or jun were found to be more susceptible to differentiation induction (Lord et al., 1993).

Within two days of LIF or IL-6 treatment, M1 cells express markers characteristic of macrophages, including βFc receptor-γ II (CD32; Lotem and Sachs, 1976). Within four days other markers, such as mac-1 (CD11b/CD18), the M-CSF/CSF-1 receptor (c-fms), and lysozyme are also expressed (Krystosek and Sachs, 1976; Liebermann and Hoffman-Liebermann, 1989; Lotem and Sachs, 1976). After four days in LIF M1 cells cease proliferation and have the morphology of mature macro-

phages, with a low nuclear to cytoplasmic ratio and numerous vacuoles (Ichikawa, 1969, 1970; Metcalf, 1989; Metcalf et al., 1988).

When cultured in agar, M1 cells form tight colonies containing 3,000-5,000 cells. In the presence of LIF, colonies are smaller and dispersed, reflecting the suppression of proliferation and the ability of mature macrophages to migrate through agar (Metcalf, 1989; Metcalf et al., 1988). The expression of the M-CSF receptor coincides with the acquisition of M-CSF responsiveness by M1 cells . The inclusion of M-CSF with LIF in cultures of M1 cells increases the viability of cells and reduces the ability of LIF to suppress clonogenicity, resulting in larger colonies in agar (Lotem and Sachs, 1992; Metcalf, 1989; Metcalf et al., 1988).

LIF also influences the behavior of the human myeloid leukemic cell lines, HL-60 and U937 (Maekawa and Metcalf, 1989; Maekawa et al., 1990). Compared to its action on M1, the effect of LIF on HL-60 and U937 is unimpressive, with a suppression of proliferation in the order of 30-40% observed, but little or no evidence of morphological differentiation.

Stimulation of Proliferation of Hemopoietic Cell Lines

DA-1a cells are absolutely dependent on exogenous growth factors for survival and proliferation and are routinely grown in IL-3. Since, however, human IL-3 is not active on murine cells, the ability of medium conditioned by human T cells to support the growth of DA-1a cells suggested the presence of a novel hemopoietic growth factor (Moreau et al., 1986, 1987). Moreau and colleagues termed this activity human interleukin for DA-1 cells (HILDA). Expression cloning and amino acid sequence analysis of HILDA showed it to be identical to human LIF (Gascan et al., 1989; Moreau et al., 1988) The factor-dependent human cell line TF-1 has also been shown to proliferate in response to LIF (Van Damme et al., 1992).

Ba/F3 cells, like DA-1a cells, are IL-3-dependent murine haemopoietic cells. In contrast to DA-1a cells, however, Ba/F3 cells do not survive or proliferate in LIF. The likely reason for this difference is the failure of Ba/F3 cells to express the LIF receptor α-chain and gp130. Stable transfection of Ba/F3 cells with plasmids directing the expression of these receptor components results in the generation of cells capable of proliferating in response to LIF (Gearing et al., 1994).

Enhancement of Megakaryocyte and Platelet Generation

The culture of primary murine and human hemopoietic progenitor cells from the bone marrow, spleen, fetal liver, or blood with LIF alone results in the generation of no colonies (Metcalf et al., 1988). LIF does, however, synergize with IL-3 in the generation of megakaryocyte colonies. The activity of LIF in enhancing megakaryocyte formation is best observed at less than optimal concentration of IL-3. For example, 50 U of IL-3 stimulates 15-20 megakaryocyte colonies in the absence of LIF, but 35-50 in the presence of 1,000 U/ml of LIF. The action of LIF in combination with IL-3 (Burstein et al., 1992; Metcalf et al., 1991) is similar to the actions of IL-6 (Debili et al., 1993; Imai et al., 1991; Koike et al., 1990; Warren et al., 1989; Williams et al., 1992) and IL-11 (Burstein et al., 1992; Yonemura et al., 1992), however, the effect is not as dramatic as the combination of SCF with IL-3 (Brugger et al., 1993; Debili et al., 1993; Imai and Nakahata, 1994; Metcalf, 1993; Tanaka et al., 1992). LIF shows no capacity to synergise with IL-3 in the generation of other colony types nor does LIF synergize with GM-CSF in the generation of megakaryocyte colonies (Metcalf et al., 1991).

The effect of LIF on megakaryocyte formation *in vitro* is also seen *in vivo*. Three systems have been used to artificially elevate LIF levels in mice. The first involved injection of purified recombinant LIF intravenously or intraperitoneally, the second engraftment of LIF producing factor-dependent haemopoietic cells and the third the generation of mice carrying a LIF transgene transcribed from an insulin promoter. While no observations regarding megakaryocytes were reported from studies on LIF transgenic mice, megakaryocyte progenitors and megakaryocytes in the bone marrow and spleen were elevated five to 10-fold in mice injected with LIF or engrafted with LIF-producing haemopoietic cells (Metcalf and Gearing, 1989; Metcalf et al., 1990). In the case of colony forming cells, both early progenitors (BFU-meg), which give rise to colonies containing large numbers of small immature megakaryocytes and late progenitors (CFU-meg), which in turn give rise to clusters of mature megakaryocytes, were elevated (Metcalf and Gearing, 1989b; Metcalf et al., 1990). Most interestingly, platelet levels were elevated by 1.5 to 2.0-fold (Metcalf and Gearing, 1989a; Metcalf et al., 1990; Waring et al., 1993). These platelets were of normal size and, using several criteria, appeared to be functional (Waring et al., 1993). In mice injected with LIF, the elevation of platelet levels occurred at doses of LIF at which other effects, such as the production of

acute phase proteins, were not observed (Metcalf et al., 1990). LIF also increases platelet levels in rabbits and in thrombocytopaenic primates (Farese et al., 1994; Moran et al., 1994).

Effects on Multipotential Progenitor Cells and Stem Cells

In addition to generating colonies containing several lineages of blood cells, primitive hemopoietic cells, known as blast colony-forming cells (blast CFCs), may also form colonies containing undifferentiated blast cells. In turn, cells within the blast colony are themselves capable of generating colonies containing several lineages when replated. The generation of blast colonies requires IL-3 in the culture. Under these conditions the first cell division of blasts-CFCs is asynchronous. The addition of LIF to such cultures synchronizes the onset of proliferation, with all blast-CFC dividing within the first three days of culture (Leary et al., 1990). This effect is also observed with SCF, IL-6, IL-11, and G-CSF (Ikebuchi et al., 1988a, 1988b; Musashi et al., 1991; Nishimoto et al., 1994; Ogawa and Clark, 1988; Tsuji et al., 1991). LIF has also been reported to enhance the survival of haemopoietic stem cells and thus their efficiency of retroviral infection *in vitro* (Dick et al., 1991; Fletcher et al., 1990, 1991a, 1991b; Verfaillie and McGlave, 1991), although this result is not universally observed (Schaafsmaet al., 1992).

An opposite effect of LIF-deficiency on stem cells has been inferred from the analyses of mice in which the production of LIF has been disrupted by homologous recombination at the LIF gene (Escary et al., 1993). These mice have reduced numbers of primitive multipotential haemopoietic precursors as measured by the day 7 spleen colony forming assay (Escary et al., 1993). These mice also have reduced numbers of committed progenitor cells in the spleen but not the bone marrow (Escary et al., 1993). The haemopoietic defects observed in these mice are clearly less dramatic than the primary phenotype, discussed in the following section—the failure of blastocysts to implant in the uterus.

Effects of LIF on Lymphoid Tissues and Lymphoid Cell

In addition to the defects in thymocyte activation observed in mice that fail to produce LIF (Escary et al., 1993), elevated expression of LIF also effects lymphopoiesis (Shen et al., 1994). Transgenic mice engineered so that T lymphocytes express LIF exhibit a number of abnormalities. These include B-cell hyperplasia with consequent

hypergammaglobulinemia and glomerulonephritis. Similar effects are observed with IL-6 transgenic mice. Additionally, LIF transgenic mice exhibit a remarkable change in the architecture of lymphoid organs. Notably, the thymus displays a lymph node like character with a disorganized epithelium, a reduction in cortical thymocyte numbers and the presence of B-cell follicles, while the peripheral lymph nodes contain elevated populations of $CD4^{+}CD8^{+}$ lymphocytes (Shenet al., 1994). While the thymic epithelium is known to produce LIF (Le et al., 1990), the relationship between these results obtained from analysis of transgenic mice (Shenet al., 1994) and the normal role of LIF is difficult to reconcile. Since, however, the LIF receptor is used by other cytokines it is possible that a structurally related cytokine may play a vital role in the regulation of lymphopoiesis.

C. ES Cells, EC Cells, Germ Cells, and Blastocyst Implantation

ES Cells

The first overt differentiative step in mammalian embryogenesis is the generation of the blastocyst from the morula. The blastocyst contains two cell layers—an outer trophectoderm and an inner cell mass (ICM). The trophectoderm and ICM have very different fates; while the trophectoderm contributes to the ectoplacental cone, giant cells, and extra-embryonic ectoderm and is intimately involved with implantation into the uterine wall (Gardner et al., 1973), the ICM gives rise to all the tissues of the fetus proper, the fetal membranes (amnion, allantois, and yolk sac) and the extra-embryonic endoderm (Gardner and Johnson, 1975; Gardner and Rossant 1979; Rossant, 1975a, 1975b, 1976). In this sense the cells of the ICM are totipotent.

The cells of the ICM may be separated from the trophectoderm and cultured *in vitro*. If cultured alone, the cells rapidly lose their totipotency and differentiate (Hogan and Tilly, 1978), however, if cells from the ICM are cultured over fibroblast feeder layers they remain undifferentiated and proliferate indefinitely (Evans and Kaufman, 1981; Magnuson et al., 1982; Martin, 1981). The resultant "cell lines" have been termed embryonic stem cells or ES cells. An important feature of ES cells is that they may be reintroduced into the blastocoel of a recipient blastocyst where they become incorporated into the host ICM. The chimaeric blastocyst may be introduced into the uterus of a foster mother where development occurs normally. Indeed, cells derived from the ES cells may be found

in all the tissues of the chimaeric embryo or mouse, including the germline (Bradley et al., 1984; Nagy et al., 1990).

The notion that a secreted regulator produced by feeder layers was responsible for maintaining the undifferentiated state of ES cells was suggested by the ability of medium conditioned by various cell lines to substitute for the feeder layer. Two groups made initial biochemical characterisation of the active protein. The first was produced by STO cells (Koopman and Cotton, 1984) and termed differentiation retarding factor (DRF) and the second by Buffalo rat liver cells (Smith and Hooper, 1987) and was named differentiation inhibitory activity (DIA) . Simultaneous to the demonstration that purified recombinant LIF was capable of maintaining ES cells in a totipotent state (Nichols et al., 1990; Pease et al., 1990; Pease and Williams, 1990; Williams et al., 1988), DIA was found to be capable of stimulating DA-1a proliferation—an action known to be ascribed to LIF (Moreau et al., 1988; Smith et al., 1988). This suggested that DIA and LIF were also identical. This was confirmed by Heath and colleagues with the demonstration that COS cells transfected with a plasmid directing LIF expression secreted a protein capable of inhibiting ES cell differentiation (Smith et al., 1988).

Remarkably, the dose of LIF necessary to inhibit the differentiation of 50% of ES cells (0.5-1.0 ng/ml) is identical to the dose required to induce the differentiation of 50% of colonies of M1 monocytic leukaemia cells (Williams et al., 1988). Unlike the action of LIF on M1 cells in which the induction of differentiation is accompanied by a reduced ability to proliferate, ES cells, at least in a period of 96 hours after LIF withdrawal, do not alter their rate of cell division (Williams et al., 1988). A combination of IL-6 and the soluble IL-6 receptor α-chain, as well as, CNTF and OSM, are also able to inhibit ES cell differentiation (Conover et al., 1993; Piquet-Pellorce et al., 1994; Rose et al., 1994; Wolf et al., 1994; Yoshida et al., 1994).

The ability of ES cells to contribute to the germline has important implications. Since ES cells may be grown indefinitely in LIF and retain their pluripotentiality *in vitro*, they may be manipulated genetically. Genetic manipulation may take the form of retroviral infection (Grez et al., 1990; Stewart et al., 1985), transfection of a plasmid which directs the expression of a transgene (Shinar et al., 1989), transfection of a yeast artificial chromosome (Strauss et al., 1993), transfection of plasmids which require integration in the proximity of an active enhancer or promoter transfection to allow transcription of a cDNA encoding a marker protein such as β-galactosidase (Friedrich and Soriano, 1991) or

transfection of a plasmid capable of homologous recombination at a specific locus in the genome. Homologous recombination may be used to correct a pre-existing mutation (Doetschman et al., 1987; Thompson et al., 1989) or to disrupt a gene of interest in various manners and therefore result in an inability to produce the protein of interest from that allele (Johnson et al., 1989; Thomas and Capecchi, 1987; Zimmer and Gruss, 1989; reviewed by Capecchi, 1989). Alternatively, homologous recombination may be used to introduce a point mutation, altering the function of the protein of interest (Ernst et al., 1994; Schwartzberg et al., 1989).

Genetically manipulated ES cells may be introduced into host blastocysts as described above. In this manner germ cells in the resultant chimaeric mice may arise from the altered ES cell (reviewed by Capecchi, 1993, 1994; Robertson, 1991). In turn the genome of the ES cell may be passed onto the next generation by mating the chimaeric mice. A proportion of the progeny will thus contain one manipulated allele of the gene of interest and one wild type allele.

Where the effect of the genetic manipulation is dominant or where a dosage effect exists these heterozygous mice may be useful. Heterozygous mice are also useful when using a variation of the strategy described above. In this case homologous recombination is used to insert a marker gene, such as β-galactosidase, downstream of the promoter of the gene of interest. Thus, wherever the promoter is active, mRNA encoding β-galactosidase will be produced and the enzyme will be synthesised. These cells may then be stained blue using the appropriate substrate. If, however, the effect of the alteration is recessive the heterozygous mice must be crossed to derive offspring that contain two manipulated alleles and no wild type homologue before the biological effect of gene deletion becomes apparent (reviewed by Capecchi, 1989, 1994; Robertson, 1991).

EC Cells

ES cells show some similarities to embryonal carcinoma (EC) cells derived primarily from germ cell tumors in 129 mice. The most important difference between ES and EC cells lies in the reduced ability of the latter to contribute to normal development, especially of the germline (Brinster, 1974; Cronmiller and Mintz, 1978; Fujii and Martin, 1983; Illmensee and Mintz, 1976; Mintz and Illmensee, 1975; Papaioannou et al., 1978, 1979; Stewart, 1982; Stewart and Mintz, 1981, 1982). EC cells in

general remain undifferentiated in the absence of a feeder layer, although they may be induced to differentiate by agents such as retinoic acid. LIF also inhibits induced differentiation of EC cell lines (Brown et al., 1992; Pruitt, 1994; Pruitt and Natoli, 1992; Takagi, 1993). P19 EC cells, for example, may be induced using various agents to differentiate into endodermal, mesodermal, and neuro-ectodermal cells. LIF was shown to be capable of blocking differentiation to mesoderm and endoderm but not neuro-ectoderm. Further studies suggest that LIF is able to block mesoderm differentiation at two separate points (Pruitt and Natoli, 1992).

Primordial Germ Cells

Murine primordial germ cells, like those of the ICM, may be cultured in vitro for long periods of time on feeder cell layers. The resulting "cell lines" appear similar to ES cell lines and like them can contribute to the formation of all tissues upon reintroduction into a host blastocyst. The survival and proliferation of primordial germ cells *in vitro* was subsequently shown to require basic fibroblast growth factor, LIF, and stem cell factor (SCF; Dolci et al., 1993; Matsui et al., 1992; Pesce et al., 1993). The importance of SCF to germ cells precursors was previously suggested by defects in the migration of primordial germ cells in steel mice, which contain mutations in the SCF gene (reviewed by Russell, 1979).

Blastocyst Implantation

Examination of LIF production in a variety of adult and neonatal mouse tissues revealed that the uterus was an abundant source (Bhatt et al., 1991; Kojima et al., 1994; Stewart et al., 1992; Yang et al., 1994). The site of LIF production within the uterus was the endometrial glands and production was regulated tightly during pregnancy. In mice, peak levels of LIF were produced on day 4 of pregnancy, concurrent with the formation of the blastocyst and prior to implantation (Bhatt et al., 1991; Croy et al., 1991). The production of LIF was independent of the presence of blastocysts and under maternal control since pseudo-pregnant mice produced LIF at a similar time after mating as did pregnant mice (Bhatt et al., 1991). In addition, mice in which implantation is naturally or experimentally delayed, also delay LIF production until just prior to implanation (Bhatt et al., 1991).

Mice that fail to produce LIF because of targeted disruption of the LIF gene develop and survive relatively normally. Male LIF-deficient mice

are fertile, however, female LIF-deficient mice never produce litters. The inability of female LIF-deficient mice to produce offspring is not due to defects in germ cells or fertilization. These mice produce normal oocytes and, when mated, morula and blastocyst stage embryos are generated normally. The defect in LIF-deficient female mice appears to be at the level of blastocyst implantation (Stewart et al., 1992).

The cellular site of LIF action, that is essential for blastocyst implantation, is not clear. Indeed, indirect evidence for actions on maternal tissue and on the blastocyst may be found. The evidence which suggests that LIF may be acting on maternal tissue comes from analyses of mice, engineered by homologous recombination, to be incapable of producing the LIF receptor α-chain or gp130—the two components of the LIF receptor. Such receptor-deficient embryos form blastocysts and implant in the uterus with the outward appearance of normality, though in both cases embryos die prematurely. An action of LIF on the blastocyst is an attractive notion since there is evidence that LIF receptors are expressed by the trophectodermal cells at this stage and in the placenta of older embryos (Hilton, Williams, and Nicola, unpublished observations).

A remaining puzzle concerns the role of LIF in ICM proliferation and differentiation. Cells of the ICM of the blastocyst are the unmanipulated equivalents of ES cells. Logic might dictate that, given LIF has a profound effect on ES cell differentiation, a similarly important role might be found in the early differentiative events of embryogenesis. The normal development of embryos, incapable of producing LIF, in a wild type maternal environment suggests that there may be no essential role of LIF during this period of development. Since embryogenesis does not, however, proceed normally in the uteri of LIF-deficient mice the development of LIF-deficient embryos in an environment completely devoid of LIF is difficult to ascertain. An alternative possibility lies in the ability of several cytokines, including LIF, OSM, and CNTF, to bind to and signal through receptors containing the LIF receptor α-chain and gp130. Indeed, as mentioned above, both OSM and CNTF are capable of suppressing the differentiation of ES cells *in vitro* and either of these proteins, or an as yet undiscovered cytokine, may regulate ICM development *in utero*.

D. Effects on Nerve and Muscle Cells

Effects on Neurotransmitter Synthesis

Patterson and colleagues demonstrated *in vitro* that non-dividing peripheral adrenergic neurons, which actively synthesise catecholami-

nes, maintain their phenotype in the absence of non-neuronal cells (Patterson and Chun, 1974). If these neurons are cultured with heart cells or glioma cells or a variety of conditioned media a transition phase was observed during which neurons expressed both catecholamines and acetylcholine and which gave way to a predomination of purely cholinergic neurons (Patterson and Chun, 1974, 1977a, 1977b; Patterson et al., 1977). The factor responsible for this change in neurotransmitter phenotype was purified from medium conditioned by heart cells and was termed cholinergic differentiation factor (CDF; Fukada, 1985). CDF was purified from newborn rat heart conditioned medium and was later shown to be the rat homologue of LIF by amino acid sequence analysis (Yamamori et al., 1989). The LIF-induced switch from an adrenergic to a cholinergic neurotransmitter phenotype is observed in neurons from a number of sources, including spinal motor neurons (Bamber et al., 1994; Banner and Patterson, 1994; Fann and Patterson, 1993; Kalberg et al., 1993; Ludlam and Kessler, 1993; Ludlam et al., 1994; Michikawa et al., 1992; Nawa et al., 1991; Shadiack et al., 1993; Sun et al., 1994; Zurn and Werren, 1994; reviewed by Patterson, 1994). The induction of a cholinergic phenotype by LIF also occurs *in vivo*. In transgenic mice which produce NGF and LIF from an insulin promoter, NGF increases the density of sympathetic innervation in the pancreas and LIF reduces the level of tyrosine hydroxylase and catecholamine synthesis, while increasing choline acetyltransferase levels (Bamber et al., 1994).

LIF also regulates the production of peptide neurotransmitters by neurons. In primary cultures of primary sympathetic neurons cells, both LIF and CNTF were found to increase the levels of mRNA encoding somatostatin, substance-P, vasoactive intestinal peptide (VIP), cholecystokinin, and enkephalin, as well as choline acetyltransferase (Fann and Patterson, 1993; Nawa et al., 1991). In addition to LIF and CNTF, other regulators also increase neuropeptide levels. IL-1, for example, induces an increase in substance-P and choline acetyltransferase levels. The induction of this change by IL-1 seems to be indirect since IL-1 also increases the level of LIF mRNA and antibodies to LIF appear able to inhibit the activity present in medium conditioned by IL-1 treated cells (Shadiack et al., 1993).

VIP levels increase in neurons that are explanted or axotomized *in vivo* as does the expression of LIF mRNA. Landis and colleagues demonstrated that LIF is produced by non-neuronal ganglionic cells and that LIF plays a central role in the increase in VIP expression (Sun et al., 1994). Antibodies to LIF, but not CNTF, were found to immunoprecipi-

tate the VIP-inducing activity in medium conditioned by sympathetic ganglia (Sun et al., 1994). VIP expression upon *in vitro* culture and axotomy were also examined in mice in which the LIF gene had been disrupted by homologous recombination. In the LIF-deficient mice, the adrenergic to cholinergic switch that is normally observed during development of the sweat glands in the foot-pad appeared normal. In contrast, the ability to up-regulate VIP expression, upon explant of ganglia or after axotomy, was reduced, though not abolished completely (Rao et al., 1993), demonstrating that LIF is a critical regulator of this event but that it is not the only regulator. Evidence also suggests that OSM, like LIF, is able to induce VIP expression (Rao et al., 1992).

Effects on Neuron Survival

LIF does not simply alter neurotransmitter phenotype, it also acts as a trophic factor for embryonic and adult neurons and their precursors. Neuronal cell death occurs *in vitro* when tissue is explanted and plays a critical role in tissue modeling during development. More neurons innervate tissues, during embryogenesis, than are ultimately required. Selection occurs because the factors that stimulate survival are present in limiting amounts—these factors are termed neurotrophic factors. Neurotrophic factors also appear to be important for neuronal survival after injury.

Neurotrophic factors include NGF and three related factors, brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4. LIF and CNTF also act as neurotrophic factors. LIF and CNTF promote the survival of both sensory and motor neurons *in vitro* (Kotzbauer et al., 1994; Kushima and Hatanaka, 1992; Murphy et al., 1993; Thaler et al., 1994).

A role for LIF in neuronal development *in vivo* is also suggested by LIF mRNA expression at various developmental stages within the spinal column and dorsal root ganglion and in regions undergoing sensory innervation (Murphy et al., 1993; Patterson and Fann, 1992; Yamamori, 1991). The LIF receptor is also expressed on developing neurons. In an elegant study, Fukada and colleagues used ^{125}I-LIF and autoradiography to examine the temporal and spatial distribution of receptors in the developing rat nervous system. Receptors were expressed by all sensory, sympathetic, and parasympathetic ganglia that were examined, but were expressed only by a subset of cells in the central nervous system (Qiu et al., 1994). The expression of receptors by these cells does not, however,

mandate an action of LIF during development, since, as described, CNTF and OSM and possibly additional cytokines utilize the high affinity LIF receptor composed of the LIF receptor α-chain and gp130.

Developmental specificity of LIF neurotrophic action has also been described. Day 16.5 embryonic, but not newborn, sensory neurons from the nodose ganglion, are supported *in vitro* by LIF, CNTF, NT-3, and NT-4, but not by NGF (Thaler et al., 1994). The reverse was found in a second study—in this case LIF and CNTF were able to promote the survival of cells from 6 day old rats but not from 21-day-old embryos (Kotzbauer et al., 1994). In both studies the expression of mRNA for receptor components including the LIF receptor α-chain, the CNTF receptor α-chain, and gp130 was similar at each phase of development. The difference in responses of ganglionic cells may therefore be due to post-translational regulation of receptor expression or alternatively, differential expression of components of the signal transduction pathway.

Compelling evidence exists that LIF plays a central role in neuronal recovery after injury. Transection of the sciatic nerve *in vivo* results in an increase in the level of LIF mRNA expression in the regions surrounding the lesion, possibly by non-neuronal cells (Banner and Patterson, 1994; Sun et al., 1994). In addition, retrograde transport of LIF has been demonstrated by sensory neurons *in vitro* and *in vivo* (Ferguson et al., 1991; Hendry et al., 1992; Ure and Campenot, 1994; Ure et al., 1992). Like LIF production, retrograde transport of LIF increases with nerve injury (Curtis et al., 1994). Following axotomy, LIF or NGF administration enhances the survival of the damaged sensory neuron bodies (Cheema et al., 1994a). Evidence exists that motoneuron survival following axotomy may also be enhanced in response to LIF (Cheema et al., 1994b). As described above, mice incapable of producing LIF exhibit a pronounced defect in neurotransmitter changes following nerve injury (Rao et al., 1993). Whether certain nerves within these mice are also more susceptible to injury or disease is not clear.

In the same manner as LIF appears to be involved in the repair of nerve cells after injury, it may also play a role in the repair of muscle. LIF mRNA levels increase in response to muscle damage and LIF infusion into the site of injury results in an increased rate of regeneration through an increase in the size of muscle fibers (Barnard et al., 1994). *In vitro*, LIF also appears to act as a chemotractant and mitogen for myoblasts (Austin and Burgess, 1991).

E. The Acute Phase Response and Infection

In response to injury and infection the levels of a series of plasma proteins are elevated—this process is termed the acute phase response (Baumann, 1989). Proteins which are elevated during this response are termed acute phase proteins and are synthesized in the liver. They include C-reactive protein, serum amyloid A, the protease inhibitors α1-antichymotrypsin, and α1-antitrypsin, fibrinogen, α1-acidglycoprotein, haptoglobin, and complement factor C3 (reviewed by Baumann, 1989).

The nature of cytokines that regulate the acute phase response has been examined primarily using *in vitro* assays. Hepatic cell lines such as HepG2 and H-35 retain the ability to increase synthesis of acute phase proteins in response to certain stimuli. Activated monocytes and keratinocytes, as well as the cell line COLO 16, were found to secrete proteins capable of acting synergistically with glucocorticoids to enhance acute phase protein synthesis by hepatic cell lines (Baumann et al., 1984, 1986, 1987a). These factors include IL-1, TNF and the hepatocyte stimulating factors (HSFs; Baumann et al., 1987a, 1987b, 1988; Prowse and Baumann, 1989). Biochemical fractionation of COLO-16 conditioned resulted in the definition of three species of HSF : HSF-I, HSF-II, and HSF-III (Baumann et al., 1987). Subsequently, HSF-1 has been shown to be identical to IL-6 (Gauldie et al., 1987, 1989) while HSF-II and HSF-III were equivalent to LIF (Baumann and Wong, 1989). IL-11, OSM, and to a lesser extent CNTF also stimulate the synthesis of acute phase proteins (Baumann and Schendel, 1991; Baumann et al., 1993; Piquet-Pellorce et al., 1994; Richards et al., 1993; Schooltink et al., 1992).

The spectrum of acute phase protein synthesis stimulated by each of these cytokines is similar, as is the degree of induction (Baumann et al., 1987; Baumann and Schendel, 1991; Baumann and Wong, 1989; Kordula et al., 1991). This functional overlap reflects the use of common receptor components, the LIF receptor alpha chain and gp130, and thus similar signal transduction pathways.

Elevation of serum LIF and IL-6 levels results in an acute phase response *in vivo* (Baumann, et al. 1993). Elevated LIF levels result in a decrease in the serum albumin concentration and an increase in the erythrocyte sedimentation rate—both classic signs of an ongoing acute phase response (Metcalf et al., 1990). More extensive analysis of the effects of IL-6 have been performed (Marinkovic et al., 1989).

Although the synthesis of LIF, IL-6, IL-11, and OSM is elevated in response to tissue damage and infection and they can induce an acute

phase response when administered *in vitro*, it is likely that in many situations only IL-6 plays a role in the physiological response. Mice that fail to produce IL-6 as a result of targeted disruption of the IL-6 gene develop normally, however, they do not efficiently resolve vaccinia virus or Listeria monocytogenes infection (Kopf et al., 1994). Part of this defect is due to a failure in the T cell-dependent antibody response, however, the acute phase response was also severely compromised in response to injury and infection. In contrast, the response to lipopolysaccharide was only moderately impaired (Kopf et al., 1994). This suggests, that while IL-6 is the central mediator of the acute phase response to certain insults, it may act in concert with other inflammatory cytokines in the response to stimuli such as LPS.

Consistent with the notion that LIF is important in the host-response to certain forms of tissue damage, LPS and cytokines such as IL-1 and TNF, lead to increased transcription of the LIF and IL-6 genes and elevate the secretion of the respective proteins (Aloisi et al., 1994; Brown et al., 1994; Ishimi et al., 1992; Lotz et al., 1992). LIF and IL-6 levels are also observed to increase in animals and patients with a wide variety of infections or other inflammatory disorders. Elevated LIF levels are found transiently in patients suffering septic shock, meningitis, vasculitis, and a variety of other inflammatory disorders such as rheumatoid arthritis (Lecron et al., 1993; Lotz et al., 1992; Taupin et al., 1992; Waring et al., 1992, 1993). Likewise, in rabbits secretion of LIF increases rapidly and markedly in response to intratracheal injection of LPS. Moreover, co-injection of LIF with LPS reduced the inflammatory response, perhaps in part by reducing the synthesis of TNF and increasing IL-6 production. LIF secretion by astrocytes is also elevated in response to LPS (Aloisi et al., 1994) and, as mentioned previously, LIF plays a central role in the response to injury within the nervous system (Banner and Patterson, 1994; Sun et al., 1994). The anti-inflammatory effect of LIF is highlighted by its protective role in lethal endotoxaemia (Alexander et al., 1992; Waring et al., 1995). Injection of C57 mice with LIF 2-24 h prior to administration of LPS resulted in increased survival. Similar effects have been observed with injections of IL-1 and TNF; LIF was found to synergize with both of these regulators (Alexander et al., 1992).

F. Adipogenesis

Certain tumors elicit a wasting syndrome in patients and in animals. This effect is termed cachexia and is thought to be mediated by cytokines

released by the tumor. TNF was the first mediator of cachexia to be described (reviewed by Tracey and Cerami, 1993, 1994). Interest has recently focused on other mediators of cachexia. Mori et al. have examined a human melanoma SEKI which induces severe cachexia in mice. SEKI-conditioned medium was found to contain a protein capable of inhibiting lipoprotein lipase activity in the 3T3-L1 adipocytic cell line. The protein, termed melanocyte-derived lipoprotein lipase inhibitor (MLPLI), was purified and analysis of the N-terminal amino acid sequence showed it to be identical to LIF (Mori et al., 1989, 1991). Similar actions of LIF are observed on primary adipocytes (Marshall, 1994). The effects of LIF on lipid metabolism parallel those of TNF, although they do not appear as marked.

Mice with experimentally elevated LIF levels also exhibit a wasting syndrome. After three days, mice injected with 2 μg of LIF every eight hours exhibit a severe reduction in body weight that is due to an almost complete loss in subcutaneous fat (Metcalf, 1990). A similar effect is observed in mice engrafted with LIF-producing cells (Metcalf and Gearing, 1989a, 1989b).

G. Osteoblasts, Osteoclasts, and Chondrocytes

Bone, once formed, is continually remodeled. Maintenance of bone mass, therefore, requires a balance between bone deposition by osteoblasts and bone resorption by osteoclasts. A number of factors have been described which activate osteoclasts, increasing their ability to resorb bone. These factors have been given the generic name osteoclast activating factors (OAFs). TNF and IL-1 both display osteoclast activating activity, as does LIF. In the case of LIF, two lines of evidence suggest that activation of osteoclasts is indirect, occurring via osteoblasts. First, osteoblasts, but not osteoclasts, express cell surface receptors for LIF. Second, LIF only enhances bone resorption in cultures containing osteoblasts and osteoclasts (Allan et al., 1990). Osteoblasts respond to LIF in a variety of ways including increased proliferation, and LIF has been reported to increase the synthesis of tissue plasminogen activator inhibitors and alkaline phosphatase (Abe et al., 1986; Allan et al., 1990; Evans et al., 1994; Hakeda et al., 1991; Ishimi et al., 1992; Noda et al., 1990; Reid et al., 1990; Rodan et al., 1990; Van Beek et al., 1993). The dramatic effect of LIF on osteoblasts is best illustrated *in vivo*. Mice engrafted with LIF-producing haemopoietic cells, which reside in the bone marrow, exhibit a dramatic phenotype with increased numbers of

osteoblasts and excessive bone deposition (Metcalf and Gearing, 1989a, 1989b; Metcalf and Nicola, 1982). Indeed bone is deposited to such an extent that the bone marrow cavity of the long bones is filled with bone and haemopoiesis is ablated (Metcalf and Gearing, 1989a, 1989b; Metcalf and Nicola, 1982).

Cartilage formation is also effected by LIF. LIF increases proteoglycan resorption in porcine cartilage (Carroll and Bell, 1993). LIF also appears to be produced by synoviocytes and chondrocytes in response to inflammatory cytokines such as IL-1 and TNF, and upon stimulation with LPS (Campbell et al., 1993; Hamilton et al., 1993; Lotz et al., 1992; Marusic et al., 1993). Strikingly, LIF levels appear elevated in the synovial fluid of patients with rheumatoid arthritis (Campbell et al., 1993; Carroll and Bell, 1993; Hamilton et al., 1993; Ishimi et al., 1992; Lotz et al., 1992; Waring et al., 1993). Given the spectrum of actions that LIF exerts on bone and cartilage formation, interest has been focused on the role that LIF might play in the pathogenesis of rheumatoid arthritis.

III. THE LIF RECEPTOR

A. Distribution and Binding Properties of the LIF Receptor

The murine LIF receptor was first characterized as the receptor for D-factor on M1 myeloid leukemia cells (Yamamoto-Yamaguchi et al., 1986). High-affinity receptors (K_D=20-100 pM) have since been detected on a variety of other cell lines (embryonal stem and carcinoma cell lines, osteoblastic cell lines, hepatic, pre-adipocyte, neuronal, and monocytic cell lines as well as normal haemopoietic, osteoblastic, hepatic, neuronal, and muscle cells; Allan et al., 1990; Godard et al., 1992; Hilton et al., 1988c, 1991a, 1992; Qiu et al., 1994; Rodan et al., 1990; Williams et al., 1988; Yamamoto-Yamaguchi et al., 1986). Among haemopoietic cell populations, LIF binding was restricted to megakaryocytes, a subpopulation of monocyte/macrophages with increasing receptor numbers as these cells matured and became activated, and a small subpopulation of lymphoid-like cells in bone marrow, spleen and thymus (Hilton et al., 1991; Metcalf et al., 1991). In cultures of mouse muscle cells, myoblasts, but not the differentiated myotubes, showed specific binding of LIF (Bower et al., 1995) and, in cultures of neural crest and dorsal root ganglion, LIF bound to the majority of sensory neurons but not Schwann cells (Hendry et al., 1992).

When injected intravenously into mice, ^{125}I-LIF exhibited a rapid initial half-life of 6-8 mins and a more prolonged second clearance phase of several hours (Hilton et al., 1991). Injected LIF clearly had access to the bone marrow as evidenced by clear labeling of megakaryocytes and osteoblasts. In the liver, parenchymal hepatocytes and, in the lung, alveolar pneumocytes were heavily labeled. In the spleen, cells of the marginal zone were intensely labeled while, in the thymus, cortical endothelial cells were labeled. Mesenchymal cells of small intestinal villi and the renal glomerular tufts were also labeled with LIF. Cells of the choroid plexus and placental trophoblasts were labeled and the role of these receptors may be to exclude LIF since little penetration of LIF into the brain or to the fetal side of the placenta was noted (Hilton et al., 1991).

LIF receptors in sensory and motor neurons appear to act as classical neurotrophic receptors in that they exhibit retrograde axonal transport. Following sciatic nerve lesion, LIF accumulated in the distal portion of the sciatic nerve and ^{125}I-LIF injected into the footpad of newborn or adult mice was specifically transported to the cell bodies of neurons in the dorsal root ganglion (Hendry et al., 1992). Production of LIF, its receptor and the level of retrograde transport of LIF were increased if the axon was crushed at a distal site (Curtis et al., 1994)

For most cell types examined *in vitro*, LIF binds to a single class of high-affinity receptor of K_D=20-100 pM (Allan et al., 1990; Godard et al., 1992; Hilton and Nicola, 1992; Hilton et al., 1988c, 1991a; Williams et al., 1988). In the mouse, only activated macrophages such as those in the peritoneal cavity of GM-CSF transgenic mice displayed an additional class of low-affinity receptor (K_D=1-2 nM). Isolated membranes, whether prepared from activated macrophages or from other cells which displayed only high-affinity receptors (e.g., hepatocytes) displayed both high- and low-affinity receptors (Hilton and Nicola, 1992). Moreover, solubilization of receptors from various cell membranes in a variety of detergents, in all cases led to the production of only low-affinity receptors (K_D=1-2 nM; Hilton and Nicola, 1992). These data suggested that specific molecular interactions were needed to maintain the high-affinity state of the LIF receptor and that, if these were broken, the default form of the LIF receptor was a low-affinity one.

Kinetic analyses of LIF binding to high- and low-affinity receptors revealed that in each case the on and off kinetics were monophasic. The association rate constants were nearly identical for high- and low-affinity receptors ($k_{on}=5\text{-}8\times10^8\ M^{-1}\ min^{-1}$) but the dissociation rate constants were much faster for low-affinity ($k_{off}=0.3\text{-}0.6\ min^{-1}$) than for high-af-

finity (k_{off}=0.0008-0.0012 min^{-1}) receptors (Hilton and Nicola, 1992). As a consequence, high-affinity receptors were more likely to be internalised (k_e=0.03 min^{-1}) than low-affinity receptors (Hilton and Nicola, 1992).

Human LIF binds to mouse and rat receptors but among human haemopoietic cells its binding appears to be relatively restricted to monocyte/macrophages. High-affinity human LIF receptors (K_D=30-200 pM) have also been detected on a variety of cell lines including melanoma, neuroblastoma, choriocarcinoma, Ewing sarcoma and colon and breast tumour cell lines. Several of these cell lines also display low-affinity receptors (K_D=1-4 nM) while other neuroblastoma and myelomonocytic (U937) cell lines were reported to express only low-affinity receptors (Godard et al., 1992).

In contrast to the situation in the mouse, one study found that low-affinity receptors displayed both a slower association rate constant ($7x10^7$ M^{-1} compared to $2x10^9$ M^{-1}) and a faster dissociation rate constant (0.2 compared to 0.008 min^{-1}) than high-affinity receptors (Godard et al., 1992). However, a separate study reported that high- and low-affinity receptors had the same association rate constant ($1\text{-}7x10^8$ M-1 min^{-1}) while confirming the difference in dissociation rate constants (0.2-0.4 vs. 0.001-0.01 min^{-1} (Layton et al., 1994b). Perhaps part of the reason for this discrepancy was that the latter authors found biphasic dissociation kinetics for both "pure" high- and low-affinity human LIF receptors and they suggested that both cases represented an equilibrium mixture of two different states of the receptor (see later).

B. Soluble LIF Receptor

Normal mouse serum was found to contain a LIF-binding protein (LBP) that inhibited the biological activity of mouse and human LIF (Layton et al., 1992). This glycoprotein of 90-100 kDa bound LIF with high specificity and bound mouse LIF with a low-affinity indistinguishable from that of the low-affinity cellular LIF receptor. In fact, purification by LIF-affinity chromatography and amino acid sequencing revealed that it was a truncated form of the authentic LIF receptor beginning at position 48 and ending prior to the transmembrane domain. In normal adult mice the levels of serum LBP were about 2μg/ml but these levels were dramatically elevated (30 to 100-fold) during pregnancy (peaking at day 12-15) and reduced in neonatal mice (Layton et al., 1994a, 1994b; Yamaguchi-Yamamoto et al., 1993). UMR osteosar-

coma cells have been reported to secrete a LBP into the extracellular matrix which binds mouse LIF with the same low-affinity as described above (Mereau et al., 1993). This protein is 140 kDa, the predicted size of the soluble LIF receptor transcript described by Gearing et al. (1991) and would be expected to retain two of the three fibronectin III domains of the mature cellular LIF receptor. It may be that further proteolytic processing of this matrix-bound form of the LIF receptor results in its release into the serum.

C. Species Cross-reactivity of LIF receptors

Whereas human LIF binds to high-affinity mouse LIF receptors with similar affinity to that of mouse LIF, mouse LIF binding to human LIF receptors is essentially undetectable. However, the mouse serum LBP revealed some curious anomalies in this one-way species cross-reactivity. Mouse LIF bound to this isolated LIF receptor with the expected low-affinity (K_D=1-3 nM) but human LIF bound with a much higher affinity (K_D=10-20 pM) and this translated to a much more potent inhibition by mouse LBP of human LIF biological activity compared to mouse LIF (about 100-fold; Layton et al., 1994b). However, whereas either mouse or human LIF could completely compete for each other's binding to mouse LBP, the dose-response of competition curves depended on whether the radioactive tracer used was mouse or human. A detailed analysis of this phenomenon revealed that when both mouse and human LIF were present they reduced each other's affinity for binding to mouse LBP by about 2 logs relative to the affinity observed when only labeled and unlabeled isologous ligands were present. This type of binding interference suggested that the binding site(s) on mouse LBP for human and mouse LIF were not identical.

In order to rationalize these observations a model was proposed for the way in which LIFs interact with their receptor ligand binding chains. The ability of mouse and human LIF to completely inhibit each other's binding to mouse LBP suggested that they shared a common binding site on the receptor (site A). However, since human LIF binds with much higher affinity than mLIF, it must have additional interaction sites on the receptor not shared with mLIF (site B). It was proposed that hLIF bound first to one site (possibly site B) on the receptor and that a receptor isomerization step then allowed interaction with the second site. Binding interference could then be explained by the effect of each type of LIF on receptor isomerization which would reduce the apparent binding affinity of the non-isologous ligand. An extension of this hypothesis to the human

proposes that the primary binding site LIF receptor is at the equivalent of site B and that a receptor isomerization step allows interaction at site A. Since mLIF lacks the binding site for site B this explains the one-way species cross-reactivity and the receptor isomerization involved in hLIF binding explains the biphasic dissociation kinetics observed even for "pure" low-affinity interactions (Layton et al., 1994a).

D. Structure of LIF Receptors

Using an expressing cloning strategy with ^{125}I-LIF and autoradiographic detection of positive COS cells, a cDNA was isolated from human placenta that specifically bound human LIF with low-affinity (K_D=1-2 nM; Gearing et al., 1991). This cDNA encoded a mature 200 kDa glycoprotein that contained a 789 amino acid extracellular domain, a 26 amino acid membrane-spanning region and a 238 amino acid cytoplasmic domain.

The extracellular domain was clearly related in amino acid sequence to other receptors of the haemopoietin family including those for growth hormone, GM-CSF, G-CSF, IL-6, erythropoietin and others (Bazan, 1990a, 1990b; Gearing et al., 1991). Each of these receptors contains a 200 amino acid domain with regions of scattered sequence identity including four cysteine residues that form two disulfide bonds and the sequence element Trp-Ser-X-Trp-Ser (Figure 2; Bazan, 1990a, 1990b). In both sequence and structure (at least for the growth hormone receptor; de Vos et al., 1992) this domain can be split into two subdomains each homologous to a fibronectin III repeat which consists of seven β-strands arranged in a barrel structure. The human LIF receptor differs from other hemopoietin receptors in containing two complete haemopoietin receptor domains as well as an immunoglobulin and three additional fibronectin III domains in its extracellular region (Gearing et al., 1991; Figure 2). The cytoplasmic portion does not contain a tyrosine kinase domain but, like many other haemopoietin receptors it contains two partially conserved sequence elements called box 1 and box 2 in the subtransmembrane position (Murakami et al., 1991). The murine LIF receptor is about 75% identical in amino acid sequence to the human receptor but, at least in the liver, alternate transcripts encode a truncated form of the receptor that lacks the membrane proximal fibronectin III domain as well as the transmembrane and cytoplasmic domains (Gearing et al., 1991; Tomida et al., 1994). This is likely to be the source of the soluble LIF-binding protein detected in mouse serum.

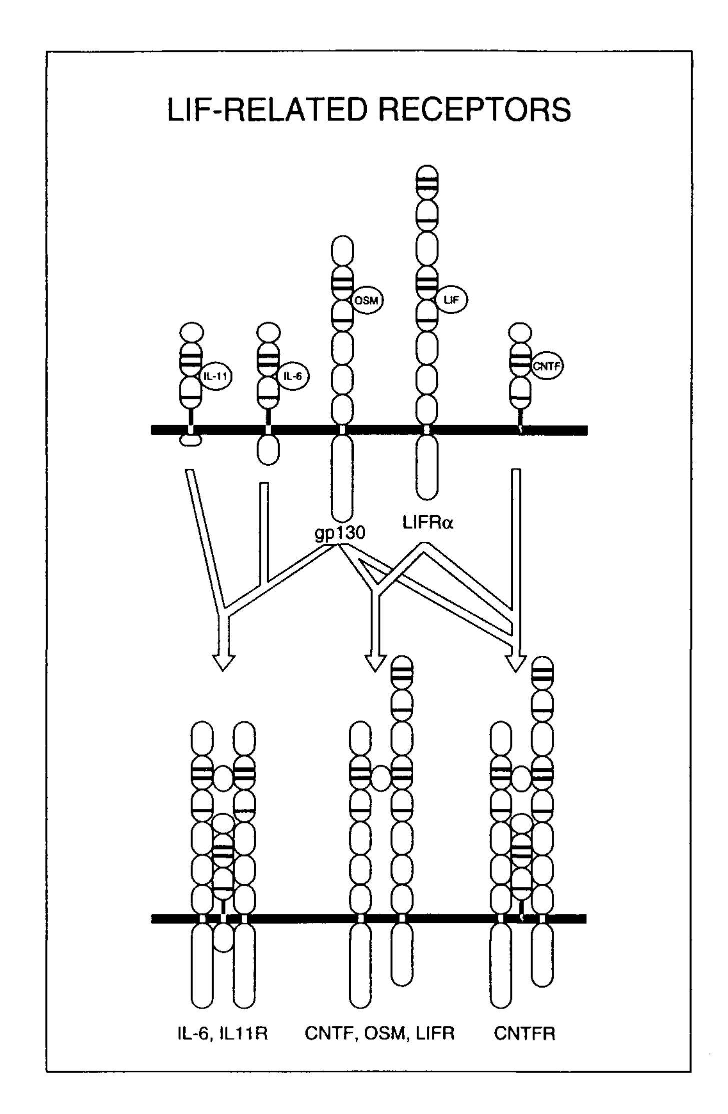


Figure 2. Structure of LIF and related receptor systems. All receptor subunits belong to the haemopoietin receptor superfamily and contain haemopoietin receptor modules (oblongs) with 4 conserved cysteines in the upper module (indicated by four lines) and the sequence WSXWS in the lower module (indicated by a heavy bar). gp130 and LIF receptor α-chain contain an additional 3 fibronectin type III modules next to the transmembrane domain, as well as immunoglobulin domains. Each ligand binds to a receptor subunit with low affinity (as seen in the upper panel). Functional receptors consist either of gp130 homodimers or LIF receptor a-chain / gp130 heterodimers with or without additional ligand specific receptor subunits as indicated in the lower panel.

The affinity of the cloned LIF receptors was typical of that of low-affinity receptors expressed on macrophages. The existence of high-affinity LIF receptors on most cell types therefore suggested the existence of a different LIF receptor or of an affinity-converting receptor subunit. Gearing and Bruce (1992) showed that a related cytokine, oncostatin M, could bind to the high-affinity LIF receptor but not to the cloned low-affinity LIF receptor. Gearing et al. (1992) reasoned that co-expression of LIF receptor with cDNAs from a placental expression library and screening for oncostatin M binding might detect the receptor components required for high-affinity LIF binding. They cloned such a receptor subunit, showed that it was identical to the affinity-converting receptor subunit for interleukin-6 (gp130), and showed that this receptor subunit bound oncostatin M with low-affinity. When co-expressed, the LIF receptor and gp130 generated high-affinity receptors for both LIF and oncostatin M implying that gp130 acted as an affinity converter for the LIF receptor and that LIF receptor acted as an affinity converter for the oncostatin M receptor, gp130 (Figure 2; Gearing and Bruce, 1992; Gearing et al., 1992).

It is now clear that the sharing of receptor subunits is not restricted to oncostatin M and LIF since gp130 is also the affinity converting subunit for interleukin-6 and interleukin-11 receptors (Figure 2; Hibi et al., 1990; Hilton et al., 1994; Murakami et al., 1993; Yin et al., 1993). On the other hand both LIF receptor and gp130 are required to form a functional receptor for ciliary neurotrophic factor (CNTF) and a ligand-specific CNTF receptor α-chain increases the affinity and responsiveness of cells that bear such receptors (Figure 2; Davis et al., 1993; Gearing et al., 1994; Ip et al., 1992; Stahl et al., 1993). The order of formation of this trimeric receptor is unclear since CNTF can bind with low-affinity to CNTFRα (K_D 10 nM), presumably to LIFR/gp130 with low-affinity, and to CNTFRα/LIFR or CNTFα/LIFR/gp130 with high-affinity (K_D 100 pM) (Gearing et al., 1994). On the other hand Davis et al. (1993) showed that CNTF could trigger the association of CNTFRα and gp130 in the absence of LIFR so there may be several routes to the formation of trimeric receptor complex.

In all cases, except for IL-11, there is now data that the functional receptor requires the association of either two gp130 molecules or one gp 130 and one LIF receptor molecule as the minimum event to allow cell signaling (Davis et al., 1993; Gearing and Bruce, 1992; Gearing et al., 1992; Murakami et al., 1993; Stahl et al., 1993). In this sense the receptor α-chains for IL-6, IL-11, CNTF, and a postulated OSM-specific

α-chain can be thought of as ligand-specific adaptors that increase the probability of dimer formation of gp130 or gp130/LIFR complexes. Indeed, it is clear at least for IL-6 and CNTF receptor α-chains that they are functional as soluble receptors with no transmembrane or cytoplasmic domains implying that dimerization is driven primarily by extracellular interactions (Panayotatos et al., 1994; Shapiro et al., 1994; Taga et al., 1989). On the other hand soluble LIF receptor and gp130 act as antagonists of cytokine action because they interfere with dimerization of the corresponding cellular receptor subunits (Layton et al., 1992; Kishimoto et al., 1992)

IV. CELL SIGNALING THROUGH LIF AND RELATED RECEPTORS

Activation of most measured cellular responses to LIF requires the heterodimerization of LIFR and gp130. The occurrence of gp130 in active IL-6, IL-11, OSM, CNTF, and cardiotrophin receptors provides a rationale for the common biological effects of the corresponding cytokines and the occurrence of LIFR/gp130 heterodimers in active OSM, CNTF, and cardiotrophin receptors suggests that the signaling pathways might be identical for these receptors. Yet each cytokine has a unique spectrum of biological activities and can have very different, if not opposite, biological effects on different cells (Table 1). In part this can be explained by the unique distributions of ligand-specific receptor α-chains among different cells which determine the potential targets a particular cytokine can access regardless of the presence of LIFR and gp 130. However, it is also likely that each receptor subunit can activate divergent cell signaling pathways and the response in any given cell will depend on its complement of signaling intermediate molecules and the way it interprets each signaling pathway (for example, by which genes are accessible for activation).

The first event in cell signaling by this family of cytokines is a ligand-induced homodimerization of gp130 or heterodimerization of gp130/LIFR. In both cases, this is driven by extracellular interactions and results in the formation, at least in part, of disulfide-linked receptor dimers (Davis et al., 1993; Murakami et al., 1993). Despite the lack of intrinsic tyrosine kinase activity in either gp130 or LIFR, there occurs shortly after ligand binding an increase in tyrosine phosphorylation of multiple intracellular substrates including gp130 and the LIFR

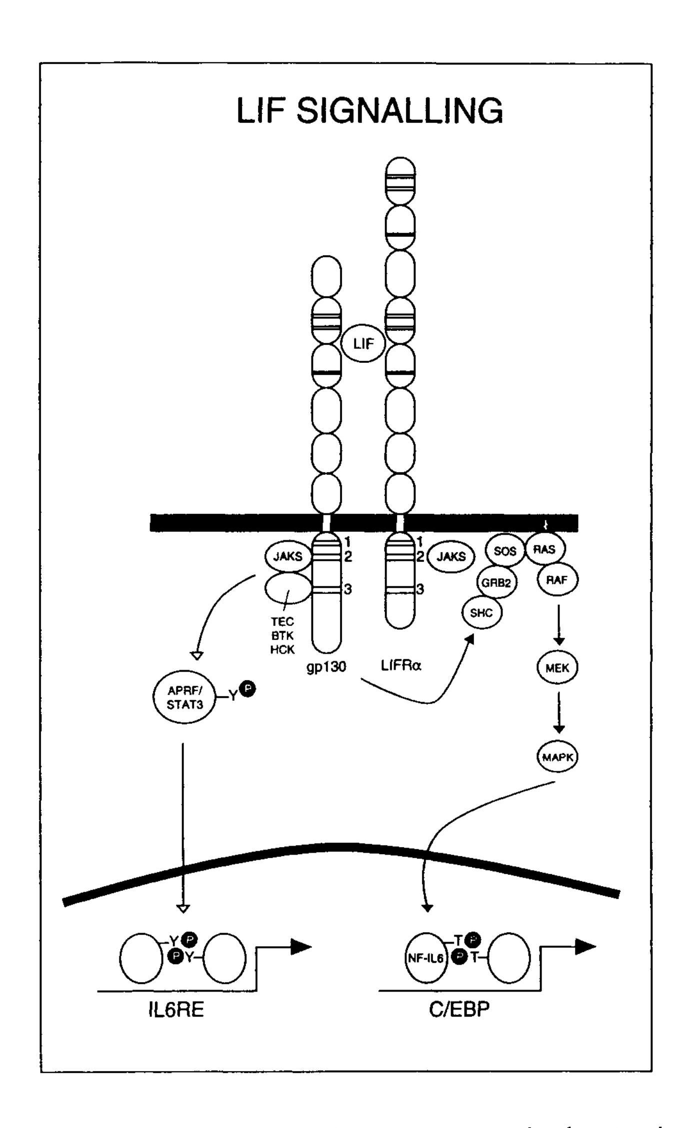


Figure 3. Cellular signaling through the LIF receptor. The functional receptor signaling complex consists of the LIF receptor α-chain and gp130. Various JAK kinases associated with these receptor subunits are bought together by ligand binding and activated by phosphorylation. In turn, APRF/STAT3 is phosphorylated and translocated to the nucleus where it binds to and activates transcription from IL-6 response elements. A different part of the gp130 cytoplasmic domain is responsible for activation of the ras/raf pathway leading to activation of MAP kinase and of the nuclear transcription factor NF-IL6.

themselves (Figure 3; Boulton et al., 1994; Davis et al., 1993; Ip et al., 1992; Lord et al., 1991; Stahl et al., 1993; Thoma et al., 1994). Other tyrosine phosphorylated proteins are common not only to the family of cytokines that utilize gp130 and LIFR but also to a large number of unrelated cytokines and growth factors, some of which utilize receptors with intrinsic tyrosine kinase activity. These tyrosine phosphorylated proteins include GRB2, SHC, raf and MAP kinases implicated in the ras-activated cell signaling pathway as well as PLCγ, PI3-kinase, and PTP1D which are involved in other distinct tyrosine kinase-activated pathways (Figure 3; Bonni et al., 1993; Boulton et al., 1994).

It might, therefore, be expected that gp130 and LIFR can recruit and activate cytoplasmic tyrosine kinases in order to activate these pathways. In embryonic stem cells it was shown that LIF binding to its receptor resulted in association and activation of hck, a haemopoietic cell-associated tyrosine kinase related to the src family (Ernst et al., 1994). In addition, members of the JAK family of cytoplasmic tyrosine kinases were demonstrated to be pre-associated with both gp130 and LIFR and became activated following ligand-induced dimerization (Figure 3). JAK kinases are unusual in that they contain two potential tyrosine kinase domains (although it is not yet clear if both are active) and a series of homology domains peculiar to the JAK family (reviewed by Wilks and Harpur, 1994). Three different JAK kinases (JAK1, JAK2, and TYK2) have been shown to associate with gp130 or LIFR or both and to be activated by ligand binding (Boulton et al., 1994; Lutticken et al., 1994; Narazaki et al., 1994; Stahl et al., 1994). The pattern of association and activation was cell type dependent but, in appropriate cells, gp130 or the heterodimer could activate all three JAK kinases. As for other cytokine receptor systems, the conserved box-1 and box-2 elements in the juxtamembrane region of the cytoplasmic domain appear to be responsible for JAK binding and activation (Stahl et al., 1994) presumably by a process of transphosphorylation of JAKs associated with different receptor subunits in the homotypic or heterotypic complexes. This unites the mechanism of activation of such receptors with that for intrinsic tyrosine kinase receptors (reviewed by Darnell et al., 1994; Ihle et al., 1994; Wilks and Harpur, 1994).

In the interferon receptor systems, activated JAKs tyrosine phosphorylate a set of related cytoplasmic proteins called signal transducers and activators of transcription (STATs; reviewed by Darnell et al., 1994). At least five such STATs have now been identified and each contains an SH2 and an SH3 domain typical of other signal transducers involved in

tyrosine kinase-activated pathways (Akira et al., 1994; Fu et al., 1992; Hou et al., 1994; Standke et al., 1994; Yamamoto et al., 1994; Zhong et al., 1994a, 1994b). The STATs may interact with activated receptor subunits or the JAKs (possibly through binding to phosphorylated tyrosines) and are then themselves phosphorylated on tyrosine by the JAKs (reviewed by Darnell et al., 1994). The STATs then dissociate from the receptor complex possibly by recognising each other's phosphorylated tyrosine through the SH2 domain, form dimers in this way, and translocate to the nucleus where they activate gene transcription (Ihle and Kerr, 1995).

LIF, CNTF, IL-6, and OSM all induce tyrosine phosphorylation and activation of a p91 similar to that induced by interferon-γ and which may be a novel STAT termed STAT3 or acute-phase response factor (APRF; Figure 3; Akira et al., 1994; Feldman et al., 1994; Lutticken et al., 1994; Wegenka et al., 1993, 1994; Yuan et al., 1994; Zhong et al., 1994a, 1994b). These activated STATs bind to DNA-enhancer sequences similar to those responsive to interferon-γ (TTC/ACNNNAA) and may be responsible for the induction of IRF-1, fos, and acute-phase protein genes (Feldman et al., 1994).

There are, however, clearly other signaling pathways that emanate from the LIF receptor. While box-1 and box-2 of the cytoplasmic domains of both gp130 and LIFR are essential for activation of the JAK-STAT pathway and sufficient for signalling cell proliferation, an additional element in the cytoplasmic domain of the LIFR (box-3, residues 141-150) is also required for activation of acute-phase protein transcription in hepatoma cells and activation of vasoactive intestinal peptide transcription in neuroblastoma cells (Baumann et al., 1994). A similar region, also required for gp130 activation (residues 110-133), is present in the cytoplasmic domain of gp130 (Baumann et al., 1994). The gp130 molecule can also activate the ras pathway leading to activation of MAP kinase, its nuclear translocation and, ultimately, the phosphorylation on Threonine 235 and activation of the transcription factor NF-IL-6 (Nakajima et al., 1993). NF-IL-6 appears to be involved in acute-phase protein expression, suppression of adipocyte differentiation and macrophage function (Akira and Kishimoto, 1992). Gene knockout experiments suggest essential roles of NF-IL-6 in G-CSF gene expression in macrophages and fibroblasts and in nitric oxide-independent intracellular bactericidal activity in macrophages (Tanaka et al., 1995).

In addition, to the activation of hck and JAK kinases in specific cells, it has been recently demonstrated that, in appropriate cells, gp130 can

also associate with and activate Btk and Tec tyrosine kinases (Matsuda et al., 1995) and also activate a different STAT-associated tyrosine kinase (p72sac; Matsuda and Hirano, 1994). Clearly, multiple tyrosine kinase pathways emanate from the LIFR and gp130 and it will be necessary to determine the cell-specific pathways and functional consequences of each of these in order to understand the molecular basis of LIF signaling.

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REFERENCES

Abe, E., Tanaka, H., Ishimi, Y., Miyaura, C., Hayashi, T., Nagasawa, H., Tomida, M., Yamaguchi, Y., Hozumi, M., & Suda, T. (1986). Differentiation-inducing factor purified from conditioned medium of mitogen-treated spleen cell cultures stimulates bone resorption. Proc. Natl. Acad. Sci. USA 83, 5958-5962.

Akira, S., & Kishimoto, T. (1992). IL-6 and NF-IL6 in acute-phase response and viral infection. Immunol Rev. 127, 25-50.

Akira, S., Nishio, Y., Inoue, M., Wang, X.J., Wei, S., Matsusaka, T., Yoshida, K., Sudo, T., Naruto, M., & Kishimoto, T. (1994). Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 77, 63-71.

Alexander, H.R., Wong, G.G., Doherty, G.M., Venzon, D.J., Fraker, D.L., & Norton, J.A. (1992). Differentiation factor/leukemia inhibitory factor protection against lethal endotoxemia in mice: Synergistic effect with interleukin 1 and tumor necrosis factor. J. Exp. Med. 175, 1139-1142.

Allan, E.H., Hilton, D.J., Brown, M.A., Evely, R.S., Yumita, S., Metcalf, D., Gough, N.M., Ng, K.W., Nicola, N.A., & Martin, T.J. (1990). Osteoblasts display receptors for and responses to leukemia-inhibitory factor. J. Cell. Physiol. 145, 110-9.

Aloisi, F., Rosa, S., Testa, U., Bonsi, P., Russo, G., Peschle, C., & Levi, G. (1994). Regulation of leukemia inhibitory factor synthesis in cultured human astrocytes. J. Immunol. 152, 5022-5031.

Anegon, I., Moreau, J.F., Godard, A., Jacques, Y., Peyrat, M.A., Hallet, M.M., Wong, G., & Soulillou, J.P. (1990). Production of human interleukin for DA cells (HILDA)/leukemia inhibitory factor (LIF) by activated monocytes. Cell. Immunol. 130, 50-65.

Austin, L., & Burgess, A.W. (1991). Stimulation of myoblast proliferation in culture by leukaemia inhibitory factor and other cytokines. J. Neurol. Sci. 101, 193-197.

Bamber, B.A., Masters, B.A., Hoyle, G.W., Brinster, R.L., & Palmiter, R.D. (1994). Leukemia inhibitory factor induces neurotransmitter switching in transgenic mice. Proc. Natl. Acad. Sci. USA 91, 7839-7843.

Banner, L.R., & Patterson, P.H. (1994). Major changes in the expression of the mRNAs for cholinergic differentiation factor/leukemia inhibitory factor and its receptor after injury to adult peripheral nerves and ganglia. Proc. Natl. Acad. Sci. USA 91, 7109-7113.

Barnard, W., Bower, J., Brown, M.A., Murphy, M., & Austin, L. (1994). Leukemia inhibitory factor (LIF) infusion stimulates skeletal muscle regeneration after injury: Injured muscle expresses lif mRNA. J. Neurol. Sci. 123, 108-113.

Barton, B.E., Jackson, J.V., Lee, F., & Wagner, J. (1994). Oncostatin M stimulates proliferation in B9 hybridoma cells: Potential role of oncostatin M in plasmacytoma development. Cytokine 6, 147-153.

Baumann, H. (1989). Hepatic acute phase reaction *in vivo* and *in vitro*. In Vitro Cell. Dev. Biol. 25, 115-126.

Baumann, H., & Schendel, P. (1991). Interleukin-11 regulates the hepatic expression of the same plasma protein genes as interleukin-6. J. Biol. Chem. 266, 20424-20427.

Baumann, H., & Wong, G.G. (1989). Hepatocyte-stimulating factor III shares structural and functional identity with leukemia-inhibitory factor. J. Immunol. 143, 1163-1167.

Baumann, H., Hill, R.E., Sauder, D.N., & Jahreis, G.P. (1986). Regulation of major acute-phase plasma proteins by hepatocyte-stimulating factors of human squamous carcinoma cells. J. Cell Biol. 102, 370-383.

Baumann, H., Jahreis, G.P., Sauder, D.N., & Koj, A. (1984). Human keratinocytes and monocytes release factors which regulate the synthesis of major acute phase plasma proteins in hepatic cells from man, rat, and mouse. J. Biol. Chem. 259, 7331-7342.

Baumann, H., Onorato, V., Gauldie, J., & Jahreis, G.P. (1987a). Distinct sets of acute phase plasma proteins are stimulated by separate human hepatocyte-stimulating factors and monokines in rat hepatoma cells. J. Biol. Chem. 262, 9756-9768.

Baumann, H., Richards, C., & Gauldie, J. (1987b). Interaction among hepatocyte-stimulating factors, interleukin 1, and glucocorticoids for regulation of acute phase plasma proteins in human hepatoma (HepG2) cells. J. Immunol. 139, 4122-4128.

Baumann, H., Prowse, K.R., Won, K.A., Marinkovic, S., & Jahreis, G.P. (1988). Regulation of acute phase protein genes by hepatocyte-stimulating factors, monokines and glucocorticoids. Tokai J. Exp. Clin. Med. 13, 277-292.

Baumann, H., Symes, A.J., Comeau, M.R., Morella, K.K., Wang, Y., Friend, D., Ziegler, S.F., Fink, J.S., & Gearing, D.P. (1994). Multiple regions within the cytoplasmic domains of the leukemia inhibitory factor receptor and gp130 cooperate in signal transduction in hepatic and neuronal cells. Mol. Cell. Biol. 14, 138-146.

Baumann, H., Ziegler, S.F., Mosley, B., Morella, K.K., Pajovic, S., & Gearing, D.P. (1993). Reconstitution of the response to leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor in hepatoma cells. J. Biol. Chem. 268, 8414-84147.

Bazan, J.F. (1990a). Haemopoietic receptors and helical cytokines. Immunol. Today 11, 350-354.
Bazan, J.F. (1990b). Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA 87, 6934-6938.
Bazan, J.F. (1991). Neuropoietic cytokines in the hematopoietic fold. Neuron 7, 197-208.
Bhatt, H., Brunet, L.J., & Stewart, C.L. (1991). Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. Proc. Natl. Acad. Sci. USA 88, 11408-11412.
Bonni, A., Frank, D.A., Schindler, C., & Greenberg, M.E. (1993). Characterization of a pathway for ciliary neurotrophic factor signalling to the nucleus. Science 262, 1575-1579.
Boulton, T.G., Stahl, N., & Yancopoulos, G.D. (1994). Ciliary neurotrophic factor/leukemia inhibitory factor/interleukin 6/oncostatin M family of cytokines induces tyrosine phosphorylation of a common set of proteins overlapping those induced by other cytokines and growth factors. J. Biol. Chem. 269, 11648-11655.
Bower, J., Vakakis, N., Nicola, N.A., & Austin, L. (1995). Specific binding of leukemia inhibitory factor to murine myoblasts in culture. J. Cell. Physiol. 164, 93-98.
Bradley, A., Evans, M., Kaufman, M.H., & Robertson, E. (1984). Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309, 255-256.
Brinster, R.L. (1974). The effect of cells transferred into the mouse blastocyst on subsequent development. J. Exp. Med. 140, 1049-1056.
Brown, G.S., Brown, M.A., Hilton, D., Gough, N.M., & Sleigh, M.J. (1992). Inhibition of differentiation in a murine F9 embryonal carcinoma cell subline by leukemia inhibitory factor (LIF). Growth Factors 7, 41-52.
Brown, M.A., Metcalf, D., & Gough, N.M. (1994). Leukaemia inhibitory factor and interleukin 6 are expressed at very low levels in the normal adult mouse and are induced by inflammation. Cytokine 6, 300-309.
Bruce, A.G., Hoggatt, I.H., & Rose, T.M. (1992). Oncostatin M is a differentiation factor for myeloid leukemia cells. J. Immunol. 149, 1271-1275.
Brugger, W., Mocklin, W., Heimfeld, S., Berenson, R.J., Mertelsmann, R., & Kanz, L. (1993). Ex vivo expansion of enriched peripheral blood CD34+ progenitor cells by stem cell factor, interleukin-1 beta (IL-1 beta), IL-6, IL-3, interferon-gamma, and erythropoietin. Blood 81, 2579-2584.
Burstein, S.A., Mei, R.L., Henthorn, J., Friese, P., & Turner, K. (1992). Leukemia inhibitory factor and interleukin-11 promote maturation of murine and human megakaryocytes in vitro. J. Cell. Physiol. 153, 305-312.
Campbell, I.K., Waring, P., Novak, U., & Hamilton, J.A. (1993). Production of leukemia inhibitory factor by human articular chondrocytes and cartilage in response to interleukin-1 and tumor necrosis factor alpha. Arth. Rheum.36, 790-794.
Capecchi, M.R. (1989). The new mouse genetics: Altering the genome by gene targeting. Trends in Genetics 5, 70-76.
Capecchi, M.R. (1993). Mouse genetics. YACs to the rescue. Nature 362, 205-206.
Capecchi, M.R. (1994). Targeted gene replacement. Sci. Am. 270, 52-59.
Carroll, G.J., & Bell, M.C. (1993). Leukaemia inhibitory factor stimulates proteoglycan resorption in porcine articular cartilage. Rheum. Int. 13, 5-8.

Cheema, S.S., Richards, L., Murphy, M., & Bartlett, P.F. (1994a). Leukemia inhibitory factor prevents the death of axotomised sensory neurons in the dorsal root ganglia of the neonatal rat. J. Neurosci. Res.37, 213-218.

Cheema, S.S., Richards, L.J., Murphy, M., & Bartlett, P.F. (1994b). Leukaemia inhibitory factor rescues motoneurones from axotomy-induced cell death. Neuroreport 5, 989-992.

Conover, J.C., Ip, N.Y., Poueymirou, W.T., Bates, B., Goldfarb, M.P., DeChiara, T.M., & Yancopoulos, G.D. (1993). Ciliary neurotrophic factor maintains the pluripotentiality of embryonic stem cells. Development 119, 559-565.

Cronmiller, C., & Mintz, B. (1978). Karyotypic normalcy and quasi-normalcy of developmentally totipotent mouse teratocarcinoma cells. Dev. Biol. 67, 465-477.

Croy, B.A., Guilbert, L.J., Browne, M.A., Gough, N.M., Stinchcomb, D.T., Reed, N., & Wegmann, T.G. (1991). Characterization of cytokine production by the metrial gland and granulated metrial gland cells. J. Repro. Immunol. 19, 149-166.

Cunningham, B.C., Ultsch, M., De Vos, A.M., Mulkerrin, M.G., Clauser, K.R., & Wells, J.A. (1991). Dimerization of the extracellular domain of the human growth hormone receptor by a single hormone molecule. Science 254, 821-825.

Curtis, R., Scherer, S.S., Somogyi, R., Adryan, K.M., Ip, N.Y., Zhu, Y., Lindsay, R.M., & DiStefano, P.S. (1994). Retrograde axonal transport of LIF is increased by peripheral nerve injury: Correlation with increased LIF expression in distal nerve. Neuron 12, 191-204.

Darnell, J., Jr., Kerr, I.M., & Stark, G.R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

Davis, S., Aldrich, T.H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., Ip, N.Y., & Yancopoulos, G.D. (1993). LIFR beta and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. Science 260, 1805-1808.

de Vos, A.M., Ultsch, M., & Kossiakoff, A.A. (1992). Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. Science 255, 306-312.

Debili, N., Masse, J.M., Katz, A., Guichard, J., Breton-Gorius, J., & Vainchenker, W. (1993). Effects of the recombinant hematopoietic growth factors interleukin-3, interleukin-6, stem cell factor, and leukemia inhibitory factor on the megakaryocytic differentiation of CD34+ cells. Blood 82, 84-95.

Derigs, H.G., & Boswell, H.S. (1993). LIF mRNA expression is transcriptionally regulated in murine bone marrow stromal cells. Leukemia 7, 630-634.

Dick, J.E., Kamel-Reid, S., Murdoch, B., & Doedens, M. (1991). Gene transfer into normal human hematopoietic cells using *in vitro* and *in vivo* assays. Blood 78, 624-34.

Doetschman, T., Gregg, R.G., Maeda, N., Hooper, M.L., Melton, D.W., Thompson, S., & Smithies, O. (1987). Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature 330, 576-578.

Dolci, S., Pesce, M., & De Felici, M. (1993). Combined action of stem cell factor, leukemia inhibitory factor, and cAMP on *in vitro* proliferation of mouse primordial germ cells. Mol. Repro. Dev.35, 134-139.

Ernst, M., Gearing, D.P., & Dunn, A.R. (1994). Functional and biochemical association of Hck with the LIF/IL-6 receptor signal transducing subunit gp130 in embryonic stem cells. EMBO J.13, 1574-1584.

Escary, J.L., Perreau, J., Dumenil, D., Ezine, S., & Brulet, P. (1993). Leukaemia inhibitory factor is necessary for maintenance of haematopoietic stem cells and thymocyte stimulation. Nature 363, 361-364.

Evans, D.B., Gerber, B., & Feyen, J.H. (1994). Recombinant human leukemia inhibitory factor is mitogenic for human bone-derived osteoblast-like cells. Biochem. Biophys. Res. Comm. 199, 220-226.

Evans, M.J., & Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. Nature 292, 154-156.

Fann, M.J., & Patterson, P.H. (1993). A novel approach to screen for cytokine effects on neuronal gene expression. J. Neurochem. 61, 1349-1355.

Farese, A.M., Myers, L.A., & MacVittie, T.J. (1994). Therapeutic efficacy of recombinant human leukemia inhibitory factor in a primate model of radiation-induced marrow aplasia. Blood 84, 3675-3678.

Feldman, G.M., Petricoin, E.3., David, M., Larner, A.C., & Finbloom, D.S. (1994). Cytokines that associate with the signal transducer gp130 activate the interferon-induced transcription factor p91 by tyrosine phosphorylation. J. Biol. Chem. 269, 10747-10752.

Ferguson, I.A., Schweitzer, J.B., Bartlett, P.F., & Johnson, E., Jr. (1991). Receptor-mediated retrograde transport in CNS neurons after intraventricular administration of NGF and growth factors. J. Comp. Neurol. 313, 680-692.

Fletcher, F.A., Moore, K.A., Ashkenazi, M., De Vries, P., Overbeek, P.A., Williams, D.E., and Belmont, J.W. (1991a). Leukemia inhibitory factor improves survival of retroviral vector-infected hematopoietic stem cells *in vitro*, allowing efficient long-term expression of vector-encoded human adenosine deaminase *in vivo*. J. Exp. Med. 174, 837-845.

Fletcher, F.A., Moore, K.A., Williams, D.E., Anderson, D., Maliszewski, C., & Belmont, J.W. (1991b). Effects of leukemia inhibitory factor (LIF) on gene transfer efficiency into murine hematolymphoid progenitors. Adv. Exp. Med. Biol. 292, 131-138.

Fletcher, F.A., Williams, D.E., Maliszewski, C., Anderson, D., Rives, M., & Belmont, J.W. (1990). Murine leukemia inhibitory factor enhances retroviral-vector infection efficiency of hematopoietic progenitors. Blood 76, 1098-1103.

Friedrich, G., & Soriano, P. (1991). Promoter traps in embryonic stem cells: A genetic screen to identify and mutate developmental genes in mice. Genes Dev. 5, 1513-1523.

Fu, X.Y., Schindler, C., Improta, T., Aebersold, R., & Darnell, J., Jr. (1992). The proteins of ISGF-3, the interferon alpha-induced transcriptional activator, define a gene family involved in signal transduction. Proc. Natl. Acad. Sci. USA 89, 7840-7843.

Fujii, J.T., & Martin, G.R. (1983). Developmental potential of teratocarcinoma stem cells *in utero* following aggregation with cleavage-stage mouse embryos. J. Emb. Exp. Morph.74, 79-96.

Fukada, K. (1985). Purification and partial characterization of a cholinergic neuronal differentiation factor. Proc. Natl. Acad. Sci. USA 82, 8795-8799.

Gardner, R.L., & Johnson, M.H. (1975). Investigation of cellular interaction and deployment in the early mammalian embryo using interspecific chimaeras between the rat and mouse. Ciba Found. Symp. 0, 183-200.

Gardner, R.L., Papaioannou, V.E., & Barton, S.C. (1973). Origin of the ectoplacental cone and secondary giant cells in mouse blastocysts reconstituted from isolated trophoblast and inner cell mass. J. Emb. Exp. Morph.30, 561-572.

Gardner, R.L., & Rossant, J. (1979). Investigation of the fate of 4-5 day post-coitum mouse inner cell mass cells by blastocyst injection. J. Emb. Exp. Morph.52, 141-152.

Gascan, H., Godard, A., Ferenz, C., Naulet, J., Praloran, V., Peyrat, M.A., Hewick, R., Jacques, Y., Moreau, J.F., & Soulillou, J.P. (1989). Characterization and NH2-terminal amino acid sequence of natural human interleukin for DA cells: Leukemia inhibitory factor. Differentiation inhibitory activity secreted by a T lymphoma cell line. J. Biol. Chem. 264, 21509-21515.

Gascan, H., & Lemetayer, J. (1991). Induction of leukemia inhibitory factor secretion by interleukin-1 in a human T lymphoma cell line. Lymph. Cyt. Res. 10, 115-118.

Gauldie, J., Richards, C., Harnish, D., Lansdorp, P., & Baumann, H. (1987). Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc. Natl. Acad. Sci. USA 84, 7251-7255.

Gauldie, J., Richards, C., Northemann, W., Fey, G., & Baumann, H. (1989). IFN beta 2/BSF2/IL-6 is the monocyte-derived HSF that regulates receptor-specific acute phase gene regulation in hepatocytes. Ann. N.Y. Acad. Sci. 557, 46-58.

Gearing, D.P., & Bruce, A.G. (1992). Oncostatin M binds the high-affinity leukemia inhibitory factor receptor. New Biologist 4, 61-65.

Gearing, D.P., Comeau, M.R., Friend, D.J., Gimpel, S.D., Thut, C.J., McGourty, J., Brasher, K.K., King, J.A., Gillis, S., Mosley, B., Ziegler, S.F., & Cosman, D. (1992). The IL-6 signal transducer, gp130: An oncostatin M receptor and affinity converter for the LIF receptor. Science 255, 1434-1437.

Gearing, D.P., Gough, N.M., King, J.A., Hilton, D.J., Nicola, N.A., Simpson, R.J., Nice, E.C., Kelso, A., & Metcalf, D. (1987). Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). EMBO J.6, 3995-4002.

Gearing, D.P., Thut, C.J., VandeBos, T., Gimpel, S.D., Delaney, P.B., King, J., Price, V., Cosman, D., & Beckmann, M.P. (1991). Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130. EMBO J.10, 2839-2848.

Gearing, D.P., Ziegler, S.F., Comeau, M.R., Friend, D., Thoma, B., Cosman, D., Park, L., & Mosley, B. (1994). Proliferative responses and binding properties of hematopoietic cells transfected with low-affinity receptors for leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor. Proc. Natl. Acad. Sci. USA 91, 1119-1123.

Gimble, J.M., Wanker, F., Wang, C.S., Bass, H., Wu, X., Kelly, K., Yancopoulos, G.D., & Hill, M.R. (1994). Regulation of bone marrow stromal cell differentiation by cytokines whose receptors share the gp130 protein. J. Cell. Biochem. 54, 122-33.

Godard, A., Gascan, H., Naulet, J., Peyrat, M.A., Jacques, Y., Soulillou, J.P., & Moreau, J.F. (1988). Biochemical characterization and purification of HILDA, a human lymphokine active on eosinophils and bone marrow cells. Blood 71, 1618-1623.

Godard, A., Heymann, D., Raher, S., Anegon, I., Peyrat, M.A., Le Mauff, B., Mouray, E., Gregoire, M., Virdee, K., Soulillou, J.P., Moreau, J-F., & Jacques, Y. (1992). High and low affinity receptors for human interleukin for DA cells/leukemia inhibitory factor on human cells. Molecular characterization and cellular distribution. J. Biol. Chem. 267, 3214-3222.

Gough, N.M., Gearing, D.P., King, J.A., Willson, T.A., Hilton, D.J., Nicola, N.A., & Metcalf, D. (1988). Molecular cloning and expression of the human homologue of the murine gene encoding myeloid leukemia-inhibitory factor. Proc. Natl. Acad. Sci. USA 85, 2623-2627.

Gough, N.M., Wilson, T.A., Stahl, J., & Brown, M.A. (1992). Molecular biology of the leukaemia inhibitory factor gene. Ciba Found. Symp. 167, 24-38.

Greenberg, A.S., Nordan, R.P., McIntosh, J., Calvo, J.C., Scow, R.O., & Jablons, D. (1992). Interleukin 6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin 6 in cancer cachexia. Canc. Res. 52, 4113-4116.

Greenfield, E.M., Gornik, S.A., Horowitz, M.C., Donahue, H.J., & Shaw, S.M. (1993). Regulation of cytokine expression in osteoblasts by parathyroid hormone: Rapid stimulation of interleukin-6 and leukemia inhibitory factor mRNA. J. Bone. Min. Res. 8, 1163-1171.

Grez, M., Akgun, E., Hilberg, F., & Ostertag, W. (1990). Embryonic stem cell virus, a recombinant murine retrovirus with expression in embryonic stem cells. Proc. Natl. Acad. Sci. USA 87, 9202-9206.

Grove, R.I., Eberhardt, C., Abid, S., Mazzucco, C., Liu, J., Kiener, P., Todaro, G., & Shoyab, M. (1993). Oncostatin M is a mitogen for rabbit vascular smooth muscle cells. Proc. Natl. Acad. Sci. USA 90, 823-827.

Hakeda, Y., Sudo, T., Ishizuka, S., Tanaka, K., Higashino, K., Kusuda, M., Kodama, H., & Kumegawa, M. (1991). Murine recombinant leukemia inhibitory factor modulates inhibitory effect of 1,25 dihydroxyvitamin D3 on alkaline phosphatase activity in MC3T3-El cells. Biochem. Biophys. Res. Comm. 175, 577-582.

Hamilton, J.A., Waring, P.M., & Filonzi, E.L. (1993). Induction of leukemia inhibitory factor in human synovial fibroblasts by IL-1 and tumor necrosis factor-alpha. J. Immunol. 150, 1496-1502.

Hendry, I.A., Murphy, M., Hilton, D.J., Nicola, N.A., & Bartlett, P.F. (1992). Binding and retrograde transport of leukemia inhibitory factor by the sensory nervous system. J. Neurosci. 12, 3427-3434.

Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T., & Kishimoto, T. (1990). Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell 63, 1149-1157.

Hilton, D.J., Hilton, A.A., Raicevic, A., Rakar, S., Harrison-Smith, M., Gough, N.M., Begley, C.G., Metcalf, D., Nicola, N.A., & Willson, T.A. (1994). Cloning of a murine IL-11 receptor alpha-chain; requirement for gp130 for high affinity binding and signal transduction. EMBO J.13, 4765-4775.

Hilton, D.J., & Nicola, N.A. (1992). Kinetic analyses of the binding of leukemia inhibitory factor to receptor on cells and membranes and in detergent solution. J. Biol. Chem. 267, 10238-10247.

Hilton, D.J., Nicola, N.A., Gough, N.M., & Metcalf, D. (1988a). Resolution and purification of three distinct factors produced by Krebs ascites cells which have

differentiation-inducing activity on murine myeloid leukemic cell lines. J. Biol. Chem. 263, 9238-9243.

Hilton, D.J., Nicola, N.A., & Metcalf, D. (1988b). Purification of a murine leukemia inhibitory factor from Krebs ascites cells. Anal. Biochem.173, 359-367.

Hilton, D.J., Nicola, N.A., & Metcalf, D. (1988c). Specific binding of murine leukemia inhibitory factor to normal and leukemic monocytic cells. Proc. Natl. Acad. Sci. USA 85, 5971-5975.

Hilton, D.J., Nicola, N.A., & Metcalf, D. (1991a). Distribution and comparison of receptors for leukemia inhibitory factor on murine hemopoietic and hepatic cells. J. Cell. Physiol. 146, 207-15.

Hilton, D.J., Nicola, N.A., Waring, P.M., & Metcalf, D. (1991b). Clearance and fate of leukemia-inhibitory factor (LIF) after injection into mice. J. Cell. Physiol. 148, 430-439.

Hilton, D.J., Nicola, N.A., & Metcalf, D. (1992). Distribution and binding properties of receptors for leukaemia inhibitory factor. Ciba Found. Symp. 167, 227-239.

Helgren, M.E., Squinto, S.P., Davis, H.L., Parry, D.J., Boulton, T.G., Heck, C.S., Zhu, Y., Yancopoulos, G.D., Lindsay, R.M., & DiStefano, P.S. (1994). Trophic effect of ciliary neurotrophic factor on denervated skeletal muscle. Cell 76, 493-504.

Hoffman-Liebermann, B., & Liebermann, D.A. (1991a). Interleukin-6- and leukemia inhibitory factor-induced terminal differentiation of myeloid leukemia cells is blocked at an intermediate stage by constitutive c-myc. Mol. Cell. Biol. 11, 2375-2381.

Hoffman-Liebermann, B., & Liebermann, D.A. (1991b). Suppression of c-myc and c-myb is tightly linked to terminal differentiation induced by IL6 or LIF and not growth inhibition in myeloid leukemia cells. Oncogene 6, 903-909.

Hogan, B., & Tilly, R. (1978). *In vitro* development of inner cell masses isolated immunosurgically from mouse blastocysts. I. Inner cell masses from 3.5-day p.c. blastocysts incubated for 24 h before immunosurgery. J. Emb. Exp. Morph.45, 93-105.

Hou, J., Schindler, U., Henzel, W.J., Ho, T.C., Brasseur, M., & McKnight, S.L. (1994). An interleukin-4-induced transcription factor: IL-4 Stat. Science 265, 1701-1706.

Hsu, L.W., & Heath, J.K. (1994). Identification of two elements involved in regulating expression of the murine leukaemia inhibitory factor gene. Biochem. J.302, 103-110.

Hughes, F.J., & Howells, G.L. (1993). Interleukin-11 inhibits bone formation *in vitro*. Calc. Tiss. Int. 53, 362-364.

Ichikawa, Y. (1969). Differentiation of a cell line of myeloid leukemia. J. Cell. Physiol. 74, 223-234.

Ichikawa, Y. (1970). Further studies on the differentiation of a cell line of myeloid leukemia. J. Cell. Physiol. 76, 175-184.

Ihle, J.N., & Kerr, I.M. (1995). JAKS and STATS in signalling by the cytokine receptor superfamily. Trends in Genetics 11, 69-74.

Ihle, J.N., Witthuhn, B.A., Quelle, F.W., Yamamoto, K., Thierfelder, W.E., Kreider, B., & Silvennoinen, O. (1994). Signaling by the cytokine receptor superfamily: JAKs and STATs. Trends. Biochem. Sci. 19, 222-227.

Ikebuchi, K., Clark, S.C., Ihle, J.N., Souza, L.M., & Ogawa, M. (1988a). Granulocyte colony-stimulating factor enhances interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. Proc. Natl. Acad. Sci. USA 85, 3445-3449.

Ikebuchi, K., Ihle, J.N., Hirai, Y., Wong, G.G., Clark, S.C., & Ogawa, M. (1988b). Synergistic factors for stem cell proliferation: further studies of the target stem cells and the mechanism of stimulation by interleukin-1, interleukin-6, and granulocyte colony-stimulating factor. Blood 72, 2007-2014.

Illmensee, K., & Mintz, B. (1976). Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocysts. Proc. Natl. Acad. Sci. USA 73, 549-553.

Imai, T., Koike, K., Kubo, T., Kikuchi, T., Amano, Y., Takagi, M., Okumura, N., & Nakahata, T. (1991). Interleukin-6 supports human megakaryocytic proliferation and differentiation in vitro. Blood 78, 1969-1974.

Imai, T., & Nakahata, T. (1994). Stem cell factor promotes proliferation of human primitive megakaryocytic progenitors, but not megakaryocytic maturation. Int. J. Hemat. 59, 91-98.

Ip, N.Y., Nye, S.H., Boulton, T.G., Davis, S., Taga, T., Li, Y., Birren, S.J., Yasukawa, K., Kishimoto, T., Anderson, D.J., Stahl, N., & Yancopoulos, G.D. (1992). CNTF and LIF act on neuronal cells via shared signaling pathways that involve the IL-6 signal transducing receptor component gp130. Cell 69, 1121-1132.

Ishimi, Y., Miyaura, C., Jin, C.H., Akatsu, T., Abe, E., Nakamura, Y., Yamaguchi, A., Yoshiki, S., Matsuda, T., Hirano, T., Kishimoto, T., & Suda, T. (1990). IL-6 is produced by osteoblasts and induces bone resorption. J. Immunol. 145, 3297-3303.

Ishimi, Y., Abe, E., Jin, C.H., Miyaura, C., Hong, M.H., Oshida, M., Kurosawa, H., Yamaguchi, Y., Tomida, M., Hozumi, M., & Suda, T. (1992). Leukemia inhibitory factor/differentiation-stimulating factor (LIF/D-factor): Regulation of its production and possible roles in bone metabolism. J. Cell. Physiol. 152, 71-78.

Johnson, R.S., Sheng, M., Greenberg, M.E., Kolodner, R.D., Papaioannou, V.E., & Spiegelman, B.M. (1989). Targeting of nonexpressed genes in embryonic stem cells via homologous recombination. Science 245, 1234-1236.

Kalberg, C., Yung, S.Y., & Kessler, J.A. (1993). The cholinergic stimulating effects of ciliary neurotrophic factor and leukemia inhibitory factor are mediated by protein kinase C. J. Neurochem. 60, 145-152.

Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O., Tanaka, H., Kuramoto, A., & Kishimoto, T. (1988). Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 332, 83-85.

Kishimoto, T., Hibi, M., Murakami, M., Narazaki, M., Saito, M., & Taga, T. (1992). The molecualr biology of IL-6 and its receptor. Ciba Found. Symp. 167, 5-16.

Koike, K., Nakahata, T., Kubo, T., Kikuchi, T., Takagi, M., Ishiguro, A., Tsuji, K., Naganuma, K., Okano, A., Akiyama, Y., & Akabane, T. (1990). Interleukin-6 enhances murine megakaryocytopoiesis in serum-free culture. Blood 75, 2286-2291.

Kojima, K., Kanzaki, H., Iwai, M., Hatayama, H., Fujimoto, M., Inoue, T., Horie, K., Nakayama, H., Fujita, J., & Mori, T. (1994). Expression of leukemia inhibitory factor in human endometrium and placenta. Biol. Reprod. 50, 882-887.

Kola, I., Davey, A., & Gough, N.M. (1990). Localization of the murine leukemia inhibitory factor gene near the centromere on chromosome 11. Growth Factors 2, 235-240.

Koopman, P., & Cotton, R.G. (1984). A factor produced by feeder cells which inhibits embryonal carcinoma cell differentiation. Characterization and partial purification. Exp. Cell Res. 154, 233-242.

Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H., & Kohler, G. (1994). Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368, 339-342.

Kordula, T., Rokita, H., Koj, A., Fiers, W., Gauldie, J., & Baumann, H. (1991). Effects of interleukin-6 and leukemia inhibitory factor on the acute phase response and DNA synthesis in cultured rat hepatocytes. Lymph. Cyt. Res. 10, 23-26.

Kotzbauer, P.T., Lampe, P.A., Estus, S., Milbrandt, J., & Johnson, E., Jr. (1994). Postnatal development of survival responsiveness in rat sympathetic neurons to leukemia inhibitory factor and ciliary neurotrophic factor. Neuron 12, 763-773.

Kreisberg, R., Detrick, M.S., & Moore, R.N. (1993). Opposing effects of tumor necrosis factor alpha and leukemia inhibitory factor in lipopolysaccharide-stimulated myelopoiesis. Inf. Immun. 61, 418-422.

Krystosek, A., & Sachs, L. (1976). Control of lysozyme induction in the differentiation of myeloid leukemic cells. Cell 9, 675-684.

Kushima, Y., & Hatanaka, H. (1992). Interleukin-6 and leukemia inhibitory factor promote the survival of acetylcholinesterase-positive neurons in culture from embryonic rat spinal cord. Neurosci. Lett. 143, 110-1104.

Layton, M.J., Cross, B.A., Metcalf, D., Ward, L.D., Simpson, R.J., & Nicola, N.A. (1992). A major binding protein for leukemia inhibitory factor in normal mouse serum: identification as a soluble form of the cellular receptor. Proc. Natl. Acad. Sci. USA 89, 8616-8620.

Layton, M.J., Lock, P., Metcalf, D., & Nicola, N.A. (1994a). Cross-species receptor binding characteristics of human and mouse leukemia inhibitory factor suggest a complex binding interaction. J. Biol. Chem. 269, 17048-17055.

Layton, M.J., Owczarek, C.M., Metcalf, D., Clark, R.L., Smith, D.K., Treutlein, H.R., & Nicola, N.A. (1994b). Conversion of the biological specificity of murine to human leukemia inhibitory factor by replacing 6 amino acid residues. J. Biol. Chem. 269, 29891-29896.

Layton, M.J., Owczarek, C.M., Metcalf, D., Lock, P.A., Wilson, T.A., Gough, N.M., Hilton, D.J., & Nicola, N.A. (1994c). Complex binding of leukemia inhibitory factor to its membrane-expressed and soluble receptors. Proc. Soc. Exp. Biol. Med. 206, 295-298.

Le, P.T., Lazorick, S., Whichard, L.P., Yang, Y.C., Clark, S.C., Haynes, B.F., & Singer, K.H. (1990). Human thymic epithelial cells produce IL-6, granulocyte-monocyte-CSF, and leukemia inhibitory factor. J. Immunol. 145, 3310-3315.

Leary, A.G., Wong, G.G., Clark, S.C., Smith, A.G., & Ogawa, M. (1990). Leukemia inhibitory factor differentiation-inhibiting activity/human interleukin for DA cells augments proliferation of human hematopoietic stem cells. Blood 75, 1960-1964.

Lecron, J.C., Roblot, P., Chevalier, S., Morel, F., Alderman, E., Gombert, J., & Gascan, H. (1993). High circulating leukaemia inhibitory factor (LIF) in patients with giant cell arteritis: independent regulation of LIF and IL-6 under corticosteroid therapy. Clin. Exp.Immunol. 92, 23-26.

Liebermann, D.A., & Hoffman-Liebermann, B. (1989). Proto-oncogene expression and dissection of the myeloid growth to differentiation developmental cascade. Oncogene 4, 583-592.

Lin, L.F., Mismer, D., Lile, J.D., Armes, L.G., Butler, E.3., Vannice, J.L., & Collins, F. (1989). Purification, cloning, and expression of ciliary neurotrophic factor (CNTF). Science 246, 1023-1025.

Lipton, J.H., & Sachs, L. (1981). Characterization of macrophage- and granulocyte-inducing proteins for normal and leukemic myeloid cells produced by the Krebs ascites tumor. Biochim. Biophys. Acta 673, 552-569.

Lord, K.A., Abdollahi, A., Hoffman-Liebermann, B., & Liebermann, D.A. (1990a). Dissection of the immediate early response of myeloid leukemia cells to terminal differentiation and growth inhibitory stimuli. Cell Growth Diff. 1, 637-645.

Lord, K.A., Abdollahi, A., Hoffman-Liebermann, B., & Liebermann, D.A. (1993). Proto-oncogenes of the fos/jun family of transcription factors are positive regulators of myeloid differentiation. Mol. Cell. Biol. 13, 841-851.

Lord, K.A., Abdollahi, A., Thomas, S.M., DeMarco, M., Brugge, J.S., Hoffman-Liebermann, B., & Liebermann, D.A. (1991). Leukemia inhibitory factor and interleukin-6 trigger the same immediate early response, including tyrosine phosphorylation, upon induction of myeloid leukemia differentiation. Mol. Cell. Biol. 11, 4371-4379.

Lord, K.A., Hoffman-Liebermann, B., & Liebermann, D.A. (1990b). Complexity of the immediate early response of myeloid cells to terminal differentiation and growth arrest includes ICAM-1, Jun-B and histone variants. Oncogene 5, 387-396.

Lord, K.A., Hoffman-Liebermann, B., & Liebermann, D.A. (1990c). Nucleotide sequence and expression of a cDNA encoding MyD88, a novel myeloid differentiation primary response gene induced by IL6. Oncogene 5, 1095-1097.

Lord, K.A., Hoffman-Liebermann, B., & Liebermann, D.A. (1990d). Sequence of MyD116 cDNA: a novel myeloid differentiation primary response gene induced by IL6. Nucl. Acids Res. 18, 2823.

Lotem, J., & Sachs, L. (1976). Control of Fc and C3 receptors on myeloid leukemic cells. J. Immunol. 117, 580-586.

Lotem, J., & Sachs, L. (1992). Regulation of leukaemic cells by interleukin 6 and leukaemia inhibitory factor. Ciba Found. Symp. 167, 80-88.

Lotem, J., Shabo, Y., & Sachs, L. (1989). Clonal variation in susceptibility to differentiation by different protein inducers in the myeloid leukemia cell line M1. Leukemia 3, 804-807.

Lotz, M., Moats, T., & Villiger, P.M. (1992). Leukemia inhibitory factor is expressed in cartilage and synovium and can contribute to the pathogenesis of arthritis. J. Clin. Invest. 90, 888-896.

Lowe, D.G., Nunes, W., Bombara, M., McCabe, S., Ranges, G.E., Henzel, W., Tomida, M., Yamamoto-Yamaguchi, Y., Hozumi, M., & Goeddel, D.V. (1989). Genomic cloning and heterologous expression of human differentiation-stimulating factor. DNA 8, 351-359.

Ludlam, W.H., & Kessler, J.A. (1993). Leukemia inhibitory factor and ciliary neurotrophic factor regulate expression of muscarinic receptors in cultured sympathetic neurons. Dev. Biol. 155, 497-506.

Ludlam, W.H., Zang, Z., McCarson, K.E., Krause, J.E., Spray, D.C., & Kessler, J.A. (1994). mRNAs encoding muscarinic and substance P receptors in cultured sympathetic neurons are differentially regulated by LIF or CNTF. Dev. Biol. 164, 528-539.

Lutticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., Taga, T., Kishimoto, T., Barbieri, G., Pellegrini, S., Sendtner, M., Heinrich, P.C., & Horn, F. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science 263, 89-92.

Maekawa, T., & Metcalf, D. (1989). Clonal suppression of HL60 and U937 cells by recombinant human leukemia inhibitory factor in combination with GM-CSF or G-CSF. Leukemia 3, 270-276.

Maekawa, T., Metcalf, D., & Gearing, D.P. (1990). Enhanced suppression of human myeloid leukemic cell lines by combinations of IL-6, LIF, GM-CSF and G-CSF. Int. J. Canc. 45, 353-358.

Magnuson, T., Epstein, C.J., Silver, L.M., & Martin, G.R. (1982). Pluripotent embryonic stem cell lines can be derived from tw5/tw5 blastocysts. Nature 298, 750-753.

Marinkovic, S., Jahreis, G.P., Wong, G.G., & Baumann, H. (1989). IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo. J. Immunol. 142, 808-812.

Marshall, M.K., Doerrler, W., Feingold, K.R., & Grunfeld, C. (1994). Leukemia inhibitory factor induces changes in lipid metabolism in cultured adipocytes. Endocrinology 135, 141-147.

Martin, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc. Natl. Acad. Sci. USA 78, 7634-7638.

Marusic, A., Kalinowski, J.F., Jastrzebski, S., & Lorenzo, J.A. (1993). Production of leukemia inhibitory factor mRNA and protein by malignant and immortalized bone cells. J. Bone. Min. Res. 8, 617-624.

Matsuda, T., & Hirano, T. (1994). Association of p72 tyrosine kinase with Stat factors and its activation by interleukin-3, interleukin-6, and granulocyte colony-stimulating factor. Blood 83, 3457-3461.

Matsuda, T., Takahashitezu, M., Fukada, T., Okuyama, Y., Fujitani, Y., Tsukada, S., Mare, H., Hirai, H., Witte, O.N., & Hirano, T. (1995). Association and activation of Btk and Tec tyrosine kinases by gp130, a signal transducer of the interleukin-6 family of cytokines. Blood 85, 627-633.

Matsui, Y., Zsebo, K., & Hogan, B.L. (1992). Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell 70, 841-847.

Mereau, A., Grey, L., Piquet-Pellorce, C., & Heath, J.K. (1993). Characterization of a binding protein for leukemia inhibitory factor localized in extracellular matrix. J. Cell Biol. 122, 713-719.

Metcalf, D. (1989). Actions and interactions of G-CSF, LIF, and IL-6 on normal and leukemic murine cells. Leukemia 3, 349-355.

Metcalf, D. (1990). The induction and inhibition of differentiation in normal and leukemic cells, Phil. Trans. Royal Soc. (London) Series B 327, 99-109.

Metcalf, D. (1993). The cellular basis for enhancement interactions between stem cell factor and the colony stimulating factors. Stem Cells 2, 1-11.

Metcalf, D., & Gearing, D.P. (1989a). Fatal syndrome in mice engrafted with cells producing high levels of the leukemia inhibitory factor. Proc. Natl. Acad. Sci. USA 86, 5948-5952.

Metcalf, D., & Gearing, D.P. (1989b). A myelosclerotic syndrome in mice engrafted with cells producing high levels of leukemia inhibitory factor (LIF). Leukemia 3, 847-852.

Metcalf, D., Hilton, D., & Nicola, N.A. (1991). Leukemia inhibitory factor can potentiate murine megakaryocyte production in vitro. Blood 77, 2150-2153.

Metcalf, D., Hilton, D.J., & Nicola, N.A. (1988). Clonal analysis of the actions of the murine leukemia inhibitory factor on leukemic and normal murine hemopoietic cells. Leukemia 2, 216-221.

Metcalf, D., & Nicola, N.A. (1982). Autoinduction of differentiation in WEHI-3B leukemia cells. Int. J. Canc. 30, 773-780.

Metcalf, D., Nicola, N.A., & Gearing, D.P. (1990). Effects of injected leukemia inhibitory factor on hematopoietic and other tissues in mice. Blood 76, 50-56.

Mezzasoma, L., Biondi, R., Benedetti, C., Floridi, C., Ciurnelli, R., Falcinelli, F., Onorato, M., Scaringi, L., Marconi, P., & Rossi, R. (1993). *In vitro* production of leukemia inhibitory factor (LIF) by Hep G2 hepatoblastoma cells. J. Biol. Reg. Homeost. Agents 7, 126-132.

Michikawa, M., Kikuchi, S., & Kim, S.U. (1992). Leukemia inhibitory factor (LIF) mediated increase of choline acetyltransferase activity in mouse spinal cord neurons in culture. Neurosci. Lett. 140, 75-77.

Miles, S.A., Martinez-Maza, O., Rezai, A., Magpantay, L., Kishimoto, T., Nakamura, S., Radka, S.F., & Linsley, P.S. (1992). Oncostatin M as a potent mitogen for AIDS-Kaposi's sarcoma-derived cells. Science 255, 1432-1434.

Miles, S.A., Rezai, A.R., Salazar-Gonzalez, J.F., Vander Meyden, M., Stevens, R.H., Logan, D.M., Mitsuyasu, R.T., Taga, T., Hirano, T., Kishimoto, T., & Martinez-Maza, O. (1990). AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. Proc. Natl. Acad. Sci. USA 87, 4068-4072.

Mintz, B., & Illmensee, K. (1975). Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc. Natl. Acad. Sci. USA 72, 3585-3589.

Moran, C.S., Campbell, J.H., Simmons, D.L., & Campbell, G.R. (1994). Human leukemia inhibitory factor inhibits development of experimental atherosclerosis. Arterio. Thromb. 14, 1356-1363.

Moreau, J.F., Bonneville, M., Peyrat, M.A., Jacques, Y., & Soulillou, J.P. (1986). Capacity of alloreactive human T clones to produce factor(s) inducing proliferation of the IL3-dependent DA-1 murine cell line. I. Evidence that this production is under IL2 control. Ann. Inst. Pasteur Immunol. 1, 25-37.

Moreau, J.F., Bonneville, M., Godard, A., Gascan, H., Gruart, V., Moore, M.A., & Soulillou, J.P. (1987). Characterization of a factor produced by human T cell clones exhibiting eosinophil-activating and burst-promoting activities. J. Immunol. 138, 3844-3849.

Moreau, J.F., Donaldson, D.D., Bennett, F., Witek-Giannotti, J., Clark, S.C., & Wong, G.G. (1988). Leukaemia inhibitory factor is identical to the myeloid growth factor human interleukin for DA cells. Nature 336, 690-692.

Mori, M., Yamaguchi, K., & Abe, K. (1989). Purification of a lipoprotein lipase-inhibiting protein produced by a melanoma cell line associated with cancer cachexia. Biochem. Biophys. Res. Comm. 160, 1085-1092.

Mori, M., Yamaguchi, K., Honda, S., Nagasaki, K., Ueda, M., Abe, O., & Abe, K. (1991). Cancer cachexia syndrome developed in nude mice bearing melanoma cells producing leukemia-inhibitory factor. Canc. Res. 51, 6656-6659.

Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T., & Kishimoto, T. (1991). Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. Proc. Natl. Acad. Sci. USA 88, 11349-11353.

Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T., & Kishimoto, T. (1993). IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. Science 260, 1808-1810.

Murphy, M., Reid, K., Brown, M.A., & Bartlett, P.F. (1993). Involvement of leukemia inhibitory factor and nerve growth factor in the development of dorsal root ganglion neurons. Development 117, 1173-1182.

Musashi, M., Yang, Y.C., Paul, S.R., Clark, S.C., Sudo, T., & Ogawa, M. (1991). Direct and synergistic effects of interleukin 11 on murine hemopoiesis in culture. Proc. Natl. Acad. Sci. USA 88, 765-769.

Nagy, A., Gocza, E., Diaz, E.M., Prideaux, V.R., Ivanyi, E., Markkula, M., & Rossant, J. (1990). Embryonic stem cells alone are able to support fetal development in the mouse. Development 110, 815-821.

Nair, B.C., DeVico, A.L., Nakamura, S., Copeland, T.D., Chen, Y., Patel, A., O'Neil, T., Oroszlan, S., Gallo, R.C., & Sarngadharan, M.G. (1992). Identification of a major growth factor for AIDS-Kaposi's sarcoma cells as oncostatin M. Science 255, 1430-1432.

Nakajima, T., Kinoshita, S., Sasagawa, T., Sasaki, K., Naruto, M., Kishimoto, T., & Akira, S. (1993). Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. Proc. Natl. Acad. Sci. USA 90, 2207-2211.

Narazaki, M., Witthuhn, B.A., Yoshida, K., Silvennoinen, O., Yasukawa, K., Ihle, J.N., Kishimoto, T., & Taga, T. (1994). Activation of JAK2 kinase mediated by the interleukin 6 signal transducer gp130. Proc. Natl. Acad. Sci. USA 91, 2285-2289.

Nawa, H., Nakanishi, S., & Patterson, P.H. (1991). Recombinant cholinergic differentiation factor (leukemia inhibitory factor) regulates sympathetic neuron phenotype by alterations in the size and amounts of neuropeptide mRNAs. J. Neurochem. 56, 2147-2150.

Nichols, J., Evans, E.P., & Smith, A.G. (1990). Establishment of germ-line-competent embryonic stem (ES) cells using differentiation inhibiting activity. Development 110, 1341-1348.

Nicola, N.A., Cross, B., & Simpson, R.J. (1993). The disulfide bond arrangement of leukemia inhibitory factor: Homology to oncostatin M and structural implications. Biochem. Biophys. Res. Comm. 190, 20-26.

Nishimoto, N., Ogata, A., Shima, Y., Tani, Y., Ogawa, H., Nakagawa, M., Sugiyama, H., Yoshizaki, K., & Kishimoto, T. (1994). Oncostatin M, leukemia inhibitory factor, and interleukin 6 induce the proliferation of human plasmacytoma cells via the common signal transducer, gp130. J. Exp. Med. 179, 1343-1347.

Noda, M., Vogel, R.L., Hasson, D.M., & Rodan, G.A. (1990). Leukemia inhibitory factor suppresses proliferation, alkaline phosphatase activity, and type I collagen messenger ribonucleic acid level and enhances osteopontin mRNA level in murine osteoblast-like (MC3T3E1) cells. Endocrin. 127, 185-190.

Ogawa, M., & Clark, S.C. (1988). Synergistic interaction between interleukin-6 and interleukin-3 in support of stem cell proliferation in culture. Blood Cells 14, 329-337.

Oh, Y.J., & O'Malley, K.L. (1994). IL-6 increases choline acetyltransferase but not neuropeptide transcripts in sympathetic neurons. Neuroreport 5, 937-940.

Owczarek, C.M., Layton, M.J., Metcalf, D., Lock, P., Willson, T.A., Gough, N.M., & Nicola, N.A. (1993). Inter-species chimeras of leukaemia inhibitory factor define a major human receptor-binding determinant. EMBO J.12, 3487-3495.

Panayotatos, N., Everdeen, D., Liten, A., Somogyi, R., & Acheson, A. (1994). Recombinant human CNTF receptor alpha: Production, binding stoichiometry, and characterization of its activity as a diffusible factor. Biochem. 33, 5813-5818.

Papaioannou, V.E., Gardner, R.L., McBurney, M.W., Babinet, C., & Evans, M.J. (1978). Participation of cultured teratocarcinoma cells in mouse embryogenesis. J. Emb. Exp. Morph.44, 93-104.

Papaioannou, V.E., Evans, E.P., Gardner, R.L., & Graham, C.F. (1979). Growth and differentiation of an embryonal carcinoma cell line (C145b). J. Emb. Exp. Morph.54, 277-295.

Patterson, P.H. (1994). Leukemia inhibitory factor, a cytokine at the interface between neurobiology and immunology. Proc. Natl. Acad. Sci. USA 91, 7833-7835.

Patterson, P.H., & Chun, L.L. (1974). The influence of non-neuronal cells on catecholamine and acetylcholine synthesis and accumulation in cultures of dissociated sympathetic neurons. Proc. Natl. Acad. Sci. USA 71, 3607-3610.

Patterson, P.H., & Chun, L.L. (1977a). The induction of acetylcholine synthesis in primary cultures of dissociated rat sympathetic neurons. I. Effects of conditioned medium. Dev. Biol. 56, 263-280.

Patterson, P.H., & Chun, L.L. (1977b). The induction of acetylcholine synthesis in primary cultures of dissociated rat sympathetic neurons. II. Developmental aspects. Dev. Biol. 60, 473-481.

Patterson, P.H., Chun, L.L., & Reichardt, L.F. (1977). The role of non-neuronal cells in the development of sympathetically derived neurons. Prog. Clin. Biol. Res. 15, 95-103.

Patterson, P.H., & Fann, M.J. (1992). Further studies of the distribution of CDF/LIF mRNA. Ciba Found. Symp. 167, 125-135.

Paul, S.R., Bennett, F., Calvetti, J.A., Kelleher, K., Wood, C.R., O'Hara, R., Jr., Leary, A.C., Sibley, B., Clark, S.C., Williams, D.A., & Yang, Y-C. (1990). Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. Proc. Natl. Acad. Sci. USA 87, 7512-7516.

Pease, S., Braghetta, P., Gearing, D., Grail, D., & Williams, R.L. (1990). Isolation of embryonic stem (ES) cells in media supplemented with recombinant leukemia inhibitory factor (LIF). Dev. Biol. 141, 344-352.

Pease, S., & Williams, R.L. (1990). Formation of germ-line chimeras from embryonic stem cells maintained with recombinant leukemia inhibitory factor. Exp. Cell Res. 190, 209-211.

Pennica, D., King, K.L., Shaw, K.J., Luis, E., Rullamas, J., Luoh, S.-M., Darbonne, W.C., Knutzon, D.S., Yen, R., Chien, K.R., Baker, J.B., & Wood, W.I. (1995). Expression cloning of cardiotrophin 1, a cytokine that induces myocyte hypertrophy. Proc. Natl. Acad. Sci. USA 92, 1142-1146.

Pesce, M., Farrace, M.G., Piacentini, M., Dolci, S., & De Felici, M. (1993). Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). Development 118, 1089-1094.

Piquet-Pellorce, C., Grey, L., Mereau, A., & Heath, J.K. (1994). Are LIF and related cytokines functionally equivalent? Exp. Cell Res. 213, 340-347.

Prowse, K.R., & Baumann, H. (1989). Interleukin-1 and interleukin-6 stimulate acute-phase protein production in primary mouse hepatocytes. J. Leuk. Biol. 45, 55-61.

Pruitt, S.C. (1994). Primitive streak mesoderm-like cell lines expressing Pax-3 and Hox gene autoinducing activities. Development 120, 37-47.

Pruitt, S.C., & Natoli, T.A. (1992). Inhibition of differentiation by leukemia inhibitory factor distinguishes two induction pathways in P19 embryonal carcinoma cells. Differentiation 50, 57-65.

Qiu, L., Bernd, P., & Fukada, K. (1994). Cholinergic neuronal differentiation factor (CDF)/leukemia inhibitory factor (LIF) binds to specific regions of the developing nervous system *in vivo*. Dev. Biol. 163, 516-520.

Rao, M.S., Symes, A., Malik, N., Shoyab, M., Fink, J.S., & Landis, S.C. (1992). Oncostatin M regulates VIP expression in a human neuroblastoma cell line. Neuroreport 3, 865-868.

Rao, M.S., Sun, Y., Escary, J.L., Perreau, J., Tresser, S., Patterson, P.H., Zigmond, R.E., Brulet, P., & Landis, S.C. (1993). Leukemia inhibitory factor mediates an injury response but not a target-directed developmental transmitter switch in sympathetic neurons. Neuron 11, 1175-1185.

Rathjen, P.D., Toth, S., Willis, A., Heath, J.K., & Smith, A.G. (1990). Differentiation inhibiting activity is produced in matrix-associated and diffusible forms that are generated by alternate promoter usage. Cell 62, 1105-1114.

Reid, L.R., Lowe, C., Cornish, J., Skinner, S.J., Hilton, D.J., Willson, T.A., Gearing, D.P., & Martin, T.J. (1990). Leukemia inhibitory factor: A novel bone-active cytokine. Endocrinology 126, 1416-1420.

Richards, C.D., Shoyab, M., Brown, T.J., & Gauldie, J. (1993). Selective regulation of metalloproteinase inhibitor (TIMP-1) by oncostatin M in fibroblasts in culture. J. Immunol. 150, 5596-5603.

Robertson, E.J. (1991). Using embryonic stem cells to introduce mutations into the mouse germ line. Biol. Reprod. 44, 238-245.

Robinson, R.C., Grey, L.M., Staunton, D., Vankelecom, H., Vernallis, A.B., Moreau, J.F., Stuart, D.I., Heath, J.K., & Jones, E.Y. (1994). The crystal structure and biological function of leukemia inhibitory factor: Implications for receptor binding. Cell 77, 1101-1116.

Rodan, S.B., Wesolowski, G., Hilton, D.J., Nicola, N.A., & Rodan, G.A. (1990). Leukemia inhibitory factor binds with high affinity to preosteoblastic RCT-1 cells and potentiates the retinoic acid induction of alkaline phosphatase. Endocrinology 127, 1602-1608.

Rose, T.M., & Bruce, A.G. (1991). Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6. Proc. Natl. Acad. Sci. USA 88, 8641-8645.

Rose, T.M., Weiford, D.M., Gunderson, N.L., & Bruce, A.G. (1994). Oncostatin M (OSM) inhibits the differentiation of pluripotent embryonic stem cells *in vitro.* Cytokine 6, 48-54.

Rossant, J. (1975a). Investigation of the determinative state of the mouse inner cell mass. I. Aggregation of isolated inner cell masses with morulae. J. Emb. Exp. Morph.33, 979-990.

Rossant, J. (1975b). Investigation of the determinative state of the mouse inner cell mass. II. The fate of isolated inner cell masses transferred to the oviduct. J. Emb. Exp. Morph.33, 991-1001.

Rossant, J. (1976). Investigation of inner cell mass determination by aggregation of isolated rat inner cell masses with mouse morulae. J. Emb. Exp. Morph. 36, 163-174.

Russell, E.S. (1979). Hereditary anemias of the mouse: a review for geneticists. Advances in Genetics 20, 357-459.

Schaafsma, M.R., Falkenburg, J.H., Duinkerken, N., Moreau, J.F., Soulillou, J.P., Willemze, R., & Fibbe, W.E. (1992). Human interleukin for DA cells (HILDA) does not affect the proliferation and differentiation of hematopoietic progenitor cells in human long-term bone marrow cultures. Exp. Hematol. 20, 6-10.

Schooltink, H., Stoyan, T., Roeb, E., Heinrich, P.C., & Rose-John, S. (1992). Ciliary neurotrophic factor induces acute-phase protein expression in hepatocytes. FEBS Lett. 314, 280-284.

Schwartzberg, P.L., Goff, S.P., & Robertson, E.J. (1989). Germ-line transmission of a c-abl mutation produced by targeted gene disruption in ES cells. Science 246, 799-803.

Selvakumaran, M., Liebermann, D.A., & Hoffman-Liebermann, B. (1992). Deregulated c-myb disrupts interleukin-6- or leukemia inhibitory factor-induced myeloid differentiation prior to c-myc: role in leukemogenesis. Mol. Cell. Biol. 12, 2493-2500.

Shabo, Y., Lotem, J., Rubinstein, M., Revel, M., Clark, S.C., Wolf, S.F., Kamen, R., & Sachs, L. (1988). The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. Blood 72, 2070-2073.

Shadiack, A.M., Hart, R.P., Carlson, C.D., & Jonakait, G.M. (1993). Interleukin-1 induces substance P in sympathetic ganglia through the induction of leukemia inhibitory factor (LIF). J. Neurosci. 13, 2601-2609.

Shapiro, L., Panayotatos, N., Meydani, S.N., Wu, D., & Dinarello, C.A. (1994). Ciliary neurotrophic factor combined with soluble receptor inhibits synthesis of proinflammatory cytokines and prostaglandin-E2 in vitro. Exp. Cell Res. 215, 51-56.

Shen, M.M., Skoda, R.C., Cardiff, R.D., Campos-Torres, J., Leder, P., & Ornitz, D.M. (1994). Expression of LIF in transgenic mice results in altered thymic epithelium and apparent interconversion of thymic and lymph node morphologies. EMBO J.13, 1375-1385.

Shinar, D., Yoffe, O., Shani, M., & Yaffe, D. (1989). Regulated expression of muscle-specific genes introduced into mouse embryonal stem cells: inverse correlation with DNA methylation. Differentiation 41, 116-126.

Smith, A.G., Heath, J.K., Donaldson, D.D., Wong, G.G., Moreau, J., Stahl, M., & Rogers, D. (1988). Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature 336, 688-690.

Smith, A.G., & Hooper, M.L. (1987). Buffalo rat liver cells produce a diffusible activity which inhibits the differentiation of murine embryonal carcinoma and embryonic stem cells. Dev. Biol. 121, 1-9.

Stahl, J., & Gough, N.M. (1993). Delineation of positive and negative control elements within the promoter region of the murine leukemia inhibitory factor (LIF) gene. Cytokine 5, 386-393.

Stahl, J., Gearing, D.P., Willson, T.A., Brown, M.A., King, J.A., & Gough, N.M. (1990). Structural organization of the genes for murine and human leukemia inhibitory factor. Evolutionary conservation of coding and non-coding regions. J. Biol. Chem. 265, 8833-8841.

Stahl, N., Davis, S., Wong, V., Taga, T., Kishimoto, T., Ip, N.Y., & Yancopoulos, G.D. (1993). Cross-linking identifies leukemia inhibitory factor-binding protein as a ciliary neurotrophic factor receptor component. J. Biol. Chem. 268, 7628-7631.

Stahl, N., Boulton, T.G., Farruggella, T., Ip, N.Y., Davis, S., Witthuhn, B.A., Quelle, F.W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J.N., & Yahcopoulos, G.D. (1994). Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. Science 263, 92-95.

Standke, G.J., Meier, V.S., & Groner, B. (1994). Mammary gland factor activated by prolactin on mammary epithelial cells and acute-phase response factor activated by interleukin-6 in liver cells share DNA binding and transactivation potential. Mol. Endocrinol. 8, 469-477.

Stewart, C.L. (1982). Formation of viable chimaeras by aggregation between teratocarcinomas and preimplantation mouse embryos. J. Emb. Exp. Morph.67, 167-179.

Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F., & Abbondanzo, S.J. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor . Nature 359, 76-79.

Stewart, C.L., Vanek, M., & Wagner, E.F. (1985). Expression of foreign genes from retroviral vectors in mouse teratocarcinoma chimaeras. EMBO J.4, 3701-3709.

Stewart, T.A., & Mintz, B. (1981). Successive generations of mice produced from an established culture line of euploid teratocarcinoma cells. Proc. Natl. Acad. Sci. USA 78, 6314-6318.

Stewart, T.A., & Mintz, B. (1982). Recurrent germ-line transmission of the teratocarcinoma genome from the METT-1 culture line to progeny *in vivo*. J. of Exp. Zool. 224, 465-469.

Strauss, W.M., Dausman, J., Beard, C., Johnson, C., Lawrence, J.B., & Jaenisch, R. (1993). Germ line transmission of a yeast artificial chromosome spanning the murine alpha 1(I) collagen locus. Science 259, 1904-1907.

Sun, Y., Rao, M.S., Zigmond, R.E., & Landis, S.C. (1994). Regulation of vasoactive intestinal peptide expression in sympathetic neurons in culture and after axotomy: the role of cholinergic differentiation factor/leukemia inhibitory factor. J. Neurobiol. 25, 415-430.

Sutherland, G.R., Baker, E., Hyland, V.J., Callen, D.F., Stahl, J., & Gough, N.M. (1989). The gene for human leukemia inhibitory factor (LIF) maps to 22q12. Leukemia 3, 9-13.

Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T., & Kishimoto, T. (1989). Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 58, 573-581.

Takagi, N. (1993). Variable X chromosome inactivation patterns in near-tetraploid murine EC x somatic cell hybrid cells differentiated *in vitro.* Genetica 88, 107-117.

Tanaka, T., Akira, S., Yoshida, K., Umemoto, M., Yoneda, Y., Shirafuji, N., Fujiwana, H., Suematsu, S., Yoshida, N., & Kishimoto, T. (1995). Targetted disruption of the NF-IL6 gene discloses its essentail role in bacterial killing and tumor cytotoxicity by macrophages. Cell 80, 353-361.

Tanaka, R., Koike, K., Imai, T., Shiohara, M., Kubo, T., Amano, Y., Komiyama, A., & Nakahata, T. (1992). Stem cell factor enhances proliferation, but not maturation, of murine megakaryocytic progenitors in serum-free culture. Blood 80, 1743-1749.

Tanigawa, T., Elwood, N., Metcalf, D., Cary, D., DeLuca, E., Nicola, N.A., & Begley, C.G. (1993). The SCL gene product is regulated by and differentially regulates cytokine responses during myeloid leukemic cell differentiation. Proc. Natl. Acad. Sci. USA 90, 7864-7868.

Taupin, J.L., Morel, D., Moreau, J.F., Gualde, N., Potaux, L., & Bezian, J.H. (1992). HILDA/LIF urinary excretion during acute kidney rejection. Transplantation 53, 655-658.

Thaler, C.D., Suhr, L., Ip, N., & Katz, D.M. (1994). Leukemia inhibitory factor and neurotrophins support overlapping populations of rat nodose sensory neurons in culture. Dev. Biol. 161, 338-344.

Thoma, B., Bird, T.A., Friend, D.J., Gearing, D.P., & Dower, S.K. (1994). Oncostatin M and leukemia inhibitory factor trigger overlapping and different signals through partially shared receptor complexes. J. Biol. Chem. 269, 6215-6222.

Thomas, K.R., & Capecchi, M.R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell 51, 503-512.

Thompson, S., Clarke, A.R., Pow, A.M., Hooper, M.L., & Melton, D.W. (1989). Germ line transmission and expression of a corrected HPRT gene produced by gene targeting in embryonic stem cells. Cell 56, 313-321.

Tomida, M., Yamamoto-Yamaguchi, Y., & Hozumi, M. (1984). Purification of a factor inducing differentiation of mouse myeloid leukemic M1 cells from conditioned medium of mouse fibroblast L929 cells. J. Biol. Chem. 259, 10978-10982.

Tomida, M., Yamamoto-Yamaguchi, Y., & Hozumi, M. (1994). Three different cDNAs encoding mouse D-factor/LIF receptor. J. Biochem. 115, 557-562.

Tomida, M., Yamamoto-Yamaguchi, Y., Hozumi, M., Okabe, T., & Takaku, F. (1986). Induction by recombinant human granulocyte colony-stimulating factor of differentiation of mouse myeloid leukemic M1 cells. Febs Lett. 207, 271-275.

Tracey, K.J., & Cerami, A. (1993). Tumor necrosis factor, other cytokines and disease. Ann. Rev. Cell Biol. 9, 317-343.

Tracey, K, & Cerami, A. (1994). Tumor necrosis factor: A pleiotropic cytokine and therapeutic target. Ann. Rev. Med. 45, 491-503.

Tsuji, K., Zsebo, K.M., & Ogawa, M. (1991). Enhancement of murine blast cell colony formation in culture by recombinant rat stem cell factor, ligand for c-kit. Blood 78, 1223-1229.

Ure, D.R., & Campenot, R.B. (1994). Leukemia inhibitory factor and nerve growth factor are retrogradely transported and processed by cultured rat sympathetic neurons. Dev. Biol. 162, 339-347.

Ure, D.R., Campenot, R.B., & Acheson, A. (1992). Cholinergic differentiation of rat sympathetic neurons in culture: Effects of factors applied to distal neurites. Dev. Biol. 154, 388-395.

Van Beek, E., Van der Wee-Pals, L., van de Ruit, M., Nijweide, P., Papapoulos, S., & Lowik, C. (1993). Leukemia inhibitory factor inhibits osteoclastic resorption, growth, mineralization, and alkaline phosphatase activity in fetal mouse metacarpal bones in culture. J. Bone. Min. Res. 8, 191-198.

Van Damme, J., Uyttenhove, C., Houssiau, F., Put, W., Proost, P., & Van Snick, J. (1992). Human growth factor for murine interleukin (IL)-9 responsive T cell lines: Co-induction with IL-6 in fibroblasts and identification as LIF/HILDA. European J. Immunol. 22, 2801-2808.

Verfaillie, C., & McGlave, P. (1991). Leukemia inhibitory factor/human interleukin for DA cells: A growth factor that stimulates the *in vitro* development of multipotential human hematopoietic progenitors. Blood 77, 263-270.

Waring, P., Wall, D., Dauer, R., Parkin, D., & Metcalf, D. (1993). The effects of leukaemia inhibitory factor on platelet function. Br. J. Haemat. 83, 80-87.

Waring, P., Wycherley, K., Cary, D., Nicola, N., & Metcalf, D. (1992). Leukemia inhibitory factor levels are elevated in septic shock and various inflammatory body fluids. J. Clin. Invest. 90, 2031-2037.

Waring, P.M., Carroll, G.J., Kandiah, D.A., Buirski, G., & Metcalf, D. (1993). Increased levels of leukemia inhibitory factor in synovial fluid from patients with rheumatoid arthritis and other inflammatory arthritides. Arth. Rheum.36, 911-915.

Waring, P.M., Waring, L.J., Billington, T., & Metcalf, D. (1995). Leukemia inhibitory factor protects against experimental lethal Escherichia coli septic shock in mice. Proc. Natl. Acad. Sci. USA 92, 1337-1341.

Warren, M.K., Conroy, L.B., & Rose, J.S. (1989). The role of interleukin 6 and interleukin 1 in megakaryocyte development. Exp. Hemat. 17, 1095-1099.

Wegenka, U.M., Buschmann, J., Lutticken, C., Heinrich, P.C., & Horn, F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. Mol. Cell. Biol. 13, 276-288.

Wegenka, U.M., Lutticken, C., Buschmann, J., Yuan, J., Lottspeich, F., Muller-Esterl, W., Schindler, C., Roeb, E., Heinrich, P.C., & Horn, F. (1994). The interleukin-6-activated acute-phase response factor is antigenically and functionally related to members of the signal transducer and activator of transcription (STAT) family. Mol. Cell. Biol. 14, 3186-3196.

Wesselingh, S.L., Levine, B., Fox, R.J., Choi, S., & Griffin, D.E. (1994). Intracerebral cytokine mRNA expression during fatal and nonfatal alphavirus encephalitis suggests a predominant type 2 T cell response. J. Immunol. 152, 1289-1297.

Wetzler, M., Talpaz, M., Lowe, D.G., Baiocchi, G., Gutterman, J.U., & Kurzrock, R. (1991). Constitutive expression of leukemia inhibitory factor RNA by human bone marrow stromal cells and modulation by IL-1, TNF-alpha, and TGF-beta. Exp.Hematol. 19, 347-351.

Wilks, A.F., & Harpur, A.G. (1994). Cytokine signal transduction and the JAK family of protein tyrosine kinases. Bioessays 16, 313-320.

Williams, N., Bertoncello, I., Jackson, H., Arnold, J., & Kavnoudias, H. (1992). The role of interleukin 6 in megakaryocyte formation, megakaryocyte development and platelet production. Ciba Found. Symp. 167, 160-170.

Williams, R.L., Hilton, D.J., Pease, S., Willson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N.A., & Gough, N.M. (1988). Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature 336, 684-687.

Willson, T.A., Metcalf, D., & Gough, N.M. (1992). Cross-species comparision of the sequence of the leukemia inhibitory factor gene and its protein. Eur. J. Biochem. 204, 21-30.

Wolf, E., Kramer, R., Polejaeva, I., Thoenen, H., & Brem, G. (1994). Efficient generation of chimaeric mice using embryonic stem cells after long-term culture in the presence of ciliary neurotrophic factor. Transgenic Res. 3, 152-158.

Yamaguchi-Yamamoto, Y., Tomida, M., & Hozumi, M. (1993). Pregnancy associated increase in differentiation-stimulating factor (D-factor)/leukemia inhibitory factor (LIF)-binding substance(s) in mouse serum. Leuk. Res. 17, 515-522.

Yamamori, T. (1991). Localization of cholinergic differentiation factor/leukemia inhibitory factor mRNA in the rat brain and peripheral tissues. Proc. Natl. Acad. Sci. USA 88, 7298-7302.

Yamamori, T., Fukada, K., Aebersold, R., Korsching, S., Fann, M.J., & Patterson, P.H. (1989). The cholinergic neuronal differentiation factor from heart cells is identical to leukemia inhibitory factor. Science 246, 1412-1416.

Yamamoto, K., Quelle, F.W., Thierfelder, W.E., Kreider, B.L., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., Silvennoinen, O., & Ihle, J.N. (1994). Stat4, a novel gamma interferon activation site-binding protein expressed in early myeloid differentiation. Mol. Cell. Biol. 14, 4342-4349.

Yamamoto, Y., Tomida, M., & Hozumi, M. (1980). Production by mouse spleen cells of factors stimulating differentiation of mouse myeloid leukemic cells that differ from the colony-stimulating factor. Cancer Res. 40, 4804-4809.

Yamamoto, Y., Tomida, M., Hozumi, M., AyUSA wa, D., Seno, T., & Tamura, G. (1981). Effect of tunicamycin on production by mouse fibroblast L929 cells of the factor-stimulating differentiation of mouse myeloid leukemic cells and the colony-stimulating factor. Cancer Research 41, 2534-2539.

Yamamoto-Yamaguchi, Y., Tomida, M., & Hozumi, M. (1986). Specific binding of a factor inducing differentiation to mouse myeloid leukemic M1 cells. Exp. Cell Res. 164, 97-102.

Yamamoto-Yamaguchi, Y., Tomida, M., Hozumi, M., Maurer, H.R., Okabe, T., & Takaku, F. (1989). Combined effects of differentiation-inducing factor and other cytokines on induction of differentiation of mouse myeloid leukemic cells. Japanese J. Cancer Res. 80, 115-121.

Yang, Z.M., Le, S.P., Chen, D.B., & Harper, M.J. (1994). Temporal and spatial expression of leukemia inhibitory factor in rabbit uterus during early pregnancy. Mol. Repro. Dev.38, 148-152.

Yin, T., Miyazawa, K., & Yang, Y.C. (1992). Characterization of interleukin-11 receptor and protein tyrosine phosphorylation induced by interleukin-11 in mouse 3T3-L1 cells. J. Biol. Chem. 267, 8347-83451.

Yin, T., Taga, T., Tsang, M.L., Yasukawa, K., Kishimoto, T., & Yang, Y.C. (1993). Involvement of IL-6 signal transducer gp130 in IL-11-mediated signal transduction. J. Immunol. 151, 2555-2561.

Yonemura, Y., Kawakita, M., Masuda, T., Fujimoto, K., Kato, K., & Takatsuki, K. (1992). Synergistic effects of interleukin 3 and interleukin 11 on murine megakaryopoiesis in serum-free culture. Exp. Hematol. 20, 1011-1016.

Yoshida, K., Chambers, I., Nichols, J., Smith, A., Saito, M., Yasukawa, K., Shoyab, M., Taga, T., & Kishimoto, T. (1994). Maintenance of the pluripotential phenotype of embryonic stem cells through direct activation of gp130 signalling pathways. Mechanisms of Development 45, 163-171.

Yuan, J., Wegenka, U.M., Lutticken, C., Buschmann, J., Decker, T., Schindler, C., Heinrich, P.C., & Horn, F. (1994). The signalling pathways of interleukin-6 and gamma interferon converge by the activation of different transcription factors which bind to common responsive DNA elements. Mol. Cell. Biol. 14, 1657-1668.

Zhong, Z., Wen, Z., & Darnell, J., Jr. (1994a). Stat3 and Stat4: Members of the family of signal transducers and activators of transcription. Proc. Natl. Acad. Sci. USA 91, 4806-4810.

Zhong, Z., Wen, Z., &Z Darnell, J., Jr. (1994b). Stat3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 264, 95-98.

Zimmer, A., & Gruss, P. (1989). Production of chimaeric mice containing embryonic stem (ES) cells carrying a homoeobox Hox 1.1 allele mutated by homologous recombination. Nature 338, 150-153.

Zurn, A.D., & Werren, F. (1994). Development of CNS cholinergic neurons *in vitro*: Selective effects of CNTF and LIF on neurons from mesencephalic cranial motor nuclei. Dev. Biol. 163, 309-315.

TUMOR NECROSIS FACTOR

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I. INTRODUCTION

It's already been a century ago that a doctor observed a so far unexplained correlation between severe infections of cancer patients and a reduction in tumor mass or even an elimination of the tumor. Consequently, the physician William B. Coley investigated preparations from gram negative and gram positive bacteria for their usability to treat cancer patients (Coley, 1893, 1896). Although these clinical trials revealed success in some cases, they were hampered by severe side effects. Nearly 100 years later, in 1975, Lloyd Old and his coworkers demonstrated the existence of an inducible serum protein which they recognized to be responsible for the induction of tumor necrosis in methylcholanthrene A-induced mouse sarcomas which was consequently called tumor necrosis factor (TNF; Carswell et al., 1975). Later, TNF was shown to be identical to cachectin, which had been independently defined as a central mediator of the shock state in gram negative sepsis (Beutler et al., 1985a; Tracey et al., 1986).

In 1984, human TNF was purified, biochemically characterized, and the TNF cDNA was cloned by different investigators (Pennica et al., 1984; Aggarwal et al., 1985a; Wang et al., 1985). In parallel, the structural and functional homology to lymphotoxin α (LTα) was detected, as well as the existence of common membrane receptors for both cytokines (Pennica et al., 1984). After molecular cloning and expression of the two TNF/LTα receptors, similar affinities of both receptors for the two cytokines was shown (Schall et al., 1990; Smith, C. A., et al., 1990). According to their approximate molecular weights, these receptors were designated as p55/p60 (type I; TR60) and p75/p80 (type II; TR80). With

the availability of TNF from recombinant sources and due to the impressive anti-tumoral effects in mouse models (Carswell et al., 1975), a number of articles both in the scientific and popular press claimed that this molecule would finally lead to a revolutionary change in cancer treatment. Years later, it became evident that the scientific community had focused too much interest on the antitumoral/cytotoxic effects of TNF, rather than realizing the extremely broad spectrum of its biological activities, for example, its immunostimulatory and proinflammatory capacity, which had led to severe systemic side effects in clinical trials (Kemeny et al., 1990; Furman et al., 1993). Interestingly, the TNF related protein LTα has never achieved such publicity, although it possesses largely identical bioactivities *in vitro.* In this book LTα is discussed in a separate chapter by Bharat Aggarwal, the present chapter will focus exclusively on TNF.

II. GENOMIC STRUCTURE

A single human gene coding for TNF is located on the short arm of chromosome 6 within the major histocompatibility gene complex. The TNF gene is found at a position about 350 kb telomeric from HLA class III genes. It has a length of about 3 kb and contains three introns (Nedwin et al., 1985; Carroll et al., 1987). Although the region coding for the 5′ end of the TNF mRNA is only separated by about 1 kb from the polyadenylation site of the LTα gene, there is clearly distinct regulation of both cytokines gene expression (Cuturi et al., 1987; Sung et al., 1988a; Kasid et al., 1990). This intergenic region contains a number of potential regulatory sites, only partially conserved among species. A potential AP-2 binding site is found in the promoter of the human TNF gene but not in that of the mouse or rabbit (Economou et al., 1989). In addition, several conserved sequences contain elements, capable to bind the nuclear transcription factor(s) κB (NFκB; Jongeneel, 1992; Kuprash, 1993). Some of these binding sites might indeed be active in parallel, as mutation of each of the five mouse sites leads to reduction in lipopolysaccharide (LPS) inducibility of less than 50% of the respective CAT constructs, but effects of these mutations are cumulative (Jongeneel, 1992).

A limited polymorphism within the human TNF gene has been determined by restriction fragment length polymorphism (Webb and Chaplin, 1990; Wilson et al., 1992). Correlation between expression of

certain TNF and HLA class II alleles has been reported (Wilson et al., 1993). The functional significance of this polymorphism is still a matter of debate. In NZW mice, however, a restriction fragment length polymorphism in the TNF gene has been demonstrated to correlate with low TNF production. Treatment of these mice with TNF significantly delays the development of autoimmune nephritis (Jacob and McDevitt, 1988), a disease to which NZW mice are predisposed. There is further evidence that in mice allelic differences in the first intron of the TNF gene have influence on TNF expression in the brain after infection with *Toxoplasma gondii*. Elevated expression of TNF mRNA was found to correlate with susceptibility to toxoplasmic encephalitis (Freund et al., 1992).

III. THE TNF MOLECULE

A 1.7 kb TNF mRNA codes for a 26 kDa protein with 233 amino acids. The amino-terminal region of this molecule consists of 76 amino acids, first believed to represent an atypically long signal sequence. This idea was consistent with the finding that soluble TNF is a 17 kDa protein consisting of 156 amino acids. Human TNF (but not the mouse homologue) is not glycosylated and contains a single intramolecular disulfide bridge (Pennica et al., 1984). Molecular weight determinations by gel electrophoresis (Aggarwal et al., 1985a), chromatography (Kunitani et al., 1988), and finally X-ray crystallography (Eck et al., 1988; Eck and Sprang, 1989; Jones et al., 1989) revealed that TNF forms stable, tightly packed trimers under physiological conditions. Treatment with detergents (Smith and Baglioni, 1987), suramin (Alzani et al., 1993), or dilution below the nanomolar range (Corti et al., 1992) results in dissociation of the molecules in 17 kDa monomers, paralleled by a loss of TNF's bioactivity. The single 17 kDa molecules are composed of two antiparallel ß-pleated sheets with antiparallel β-strands, forming a ß-sandwich structure. Such typical "jelly roll" β-structures have been found in a number of cytokines but also in viral capsid proteins (for review see Bazan, 1993).

Bioactive, soluble 51 kDa TNF trimers form molecules with a threefold vertical symmetry showing a tapering peak and standing on a broad base. Both C- and N-termini are at or near this platform. The trimer is held together by hydrophobic interactions of one of the β-sheeted pleats, whereas the other more hydrophilic sheet of each monomer, is directed toward the outer surface of the TNF trimer. Recently, the complex

between LTα—which forms very similar trimers as TNF does (Eck et al., 1992)—and one of the TNF receptor extracellular domains (TR60) has been analyzed by X-ray crystallography (Banner et al., 1993). These data show that receptor/ligand interaction occurs mainly at the grooves between the LTα monomers, which might explain that TNF and LTα monomers show no detectable bioactivity.

These findings, together with data from antibody mapping and mutational studies, led to construction of TNF muteins, able to specifically interact with only one of the two TNF receptors (Loetscher et al., 1993; Van Ostade et al., 1994). Amino acid exchanges on only two positions were sufficient to restrict high affinity interaction to either TR60 (Arg^{32} ⇒ Trp and Ser^{86} ⇒ Thr) or TR80 (Asp^{143} ⇒ Asn and Ala^{145} ⇒ Arg; Loetscher et al., 1993). More extensive use of these TNF muteins will reveal additional information regarding the functional role of the two TNF receptors (Van Ostade et al., 1993; Barbara et al., 1994; Van Zee et al., 1994).

The intersubunit space along the threefold axis of the 51 kDa trimer forms an about 12 Å funnel, surrounded with charged and polar amino acid side chains. Toward the platform of the trimer, the funnel becomes more narrow. It has been shown that TNF can (at least at high concentrations) integrate into membranes and then form hydrophilic pores (Baldwin et al., 1988; Kagan et al., 1992). It has been hypothesized that the capability of TNF to aggregate could then amplify this effect leading to a massive ion permeability across the respective membrane (Mirzabekov et al., 1994).

A further activity of TNF, not mediated via receptor binding, can be linked to the sugar binding capability, that is, lectin-like activity, of this molecule (Muchmore et al., 1990; Lucas et al., 1994). Due to this property, TNF possesses a direct trypanolytic activity (Kongshavn and Ghadirian, 1988) and induces egg-laying in schistosomes (Amiri et al., 1992). Further, it has been proposed that the affinity of TNF for high mannose proteins might affect kinetics of clearance (Sherblom et al., 1988). The recently described specific TNF binding capacity of gram negative bacteria, possibly affecting their virulence, might also be linked to the sugar binding capability of TNF (Luo et al., 1993).

More than four years after molecular cloning of TNF, it became evident that the first 76 amino acids encoded by the 1.7 kb TNF mRNA in fact represent a transmembrane and a cytoplasmic domain rather than a signal peptide. Accordingly, TNF is first expressed as a 26 kDa

transmembrane protein in the respective producer cells (Kriegler et al., 1988). Membrane integrated TNF shows bioactivity (Perez et al., 1990) and might mediate cytotoxic effects of $CD4^+$ T lymphocytes (Tite, 1990; Smyth and Ortaldo, 1993) and macrophages (Peck et al., 1989). Soluble 17 kDa TNF is a product thereof, proteolytically processed by a so far undefined membrane-expressed proteinase which cleaves TNF preferentially at the Ala-Val residues at positions 76-77 of the transmembrane 26 kDa molecule (Perez et al., 1990). However, additional cleavage sites exist within the first 12 amino acids of the 17 kDa TNF molecule, leading to the release of smaller TNF molecules. In addition, in both mouse (Cseh and Beutler, 1989) and man (Müller et al., 1986) an about 18.5 kDa TNF molecule has been described, possessing an additional 10 amino acids at the amino-terminus. There is good evidence that this TNF variant lacks bioactivity. Deletion of the above mentioned amino acids 77-89 of the 26 kDa product results in membrane expression of a bioactive TNF form without detectable production of soluble, 17 kDa form of TNF. Cells expressing such uncleavable TNF variants have been used to investigate the bioactivity of membrane bound TNF in detail (Perez et al., 1990; Grell et al., 1995).

Very recent data indicate that membrane-integrated TNF itself might also act as a signaling molecule. *In vivo* activation of T cells in mice with anti-CD3 antibodies could be enhanced by TNF-specific antibodies, but not by high doses of (monomeric) soluble TNF receptors (Ferran et al., 1994). These data suggest a retrograde signaling capacity of membrane integrated TNF upon appropriate cross-linking.

IV. TNF PRODUCTION

A. TNF Production *In Vitro*

Typical TNF producers are cells of the immune system. Cells of the monocyte/macrophage lineage are considered to represent the main sources (Carswell et al., 1975; Männel et al., 1980). Peripheral blood monocytes, alveolar macrophages, Kupffer cells, astrocytes, tumor infiltrating macrophages, and additional types of tissue macrophages have all been shown to secrete TNF. Other cells of the myeloid lineage like neutrophils, basophils, and mast cells are also capable of TNF production (Djeu, 1992). Further, NK cells (Peters et al., 1986) and all cells of the lymphocytic lineage can produce TNF upon appropriate stimulation,

independently regulated from LTα production (Cuturi et al., 1987; Andersson et al., 1989; Sung et al., 1988b). In most cases, however, it is unclear whether these cells mainly express the transmembrane form of the cytokine, able to exert cellular effects in a juxtacrine situation, or whether they are important producers of the soluble cytokine. Although highly sensitive for TNF themselves, endothelial cells are also capable of producing this cytokine (Jevnikar et al., 1991), a mechanism believed to be important in regulation of the hemostatic properties of endothelium and in modulation of neutrophil adhesion and activity (Schmidt et al., 1995). A surprisingly large number of nonhematopoietic tumor cell lines are TNF producers (Krönke et al., 1988), a phenomenon which has been correlated with these cells' ability to gain TNF resistance (Rubin et al., 1986).

B. TNF Production *In Vivo*

Although quantification of TNF *in vivo* is limited by the facts described in the following text, it is clear that healthy individuals have only very low if any free bioactive TNF in their serum (≤ 50 pg/ml). Challenge with LPS results in a rapid increase in TNF levels peaking after about 2 h, returning to background levels after a few additional hours (Michie et al., 1988). UV irradiation (Kock et al., 1990) and a large number of infectious agents have been shown to induce TNF production (for review see Tracey and Cerami, 1993). These include gram negative bacteria, mycobacteria, and parasites. Of great importance might be the local induction of TNF in the brain, as this cytokine is unable to pass the blood-brain barrier but is involved in brain regulation of acute phase response. Perivascular cells and neurons in circumventricular organs have both been demonstrated by *in situ* hybridization to express TNF mRNA after systemic application of LPS (Breder et al., 1994).

C. Regulation of TNF Production

A number of different agents, especially LPS, stimulate TNF secretion of monocytes *in vitro*. Various cytokines have been described to modulate LPS-induced TNF production. Stimulatory activity has been found for TNF itself, IFNγ, IL-1, IL-3, M-CSF, and GM-CSF (Warren and Ralph, 1986; Gifford and Lohmann-Matthes, 1987; Cannistra et al., 1988), whereas TGFβ inhibits LPS-induced

TNF production (Espevik et al., 1988; Chantry et al., 1989). The protein kinase C activator and tumor promoter phorbol myristate acetate is a strong stimulus for TNF synthesis, often used in *in vitro* experiments. Control of TNF production resides both at the level of transcription and at several post-transcriptional levels. Although a large number of data have been gathered during the past few years, our understanding on the function of the TNF promoter is still very rudimentary (Jongeneel, 1992; see preceding). There is good evidence for control of TNF production at the level of mRNA abundance and translation (Sariban et al., 1988; Beutler et al., 1989). Moreover, it is conceivable that the activity of the membrane proteinase(s) which process membrane-integrated 26 kDa TNF to release the soluble cytokine is a regulated process.

V. TNF ASSAY SYSTEMS

The classical assay system for determination of bioactive TNF is based on the cytotoxic effect of this cytokine for the mouse fibrosarcoma cell line L929 (Meager et al., 1989; Nargi and Yang, 1993). One unit of TNF induces half maximum cytotoxicity under standardized conditions and equals to about 20 pg/ml. This assay system will give information about the overall TNF bioactivity in a given biological fluid, although it is limited by the following facts:

1. LTα has a similar cytotoxic effect for L929 cells as compared to TNF (Meager et al., 1989). However, using TNF or LTα specific neutralizing antibodies one can distinguish between these two related cytokines.
2. This assay system might not detect the total amount of bioactive TNF (plus LTα), as the respective biofluid might also contain TNF inhibitors (shedded soluble extracellular domains of the TNF receptors), capable to compete with the L929 cells TNF receptors for TNF binding (see following).

A large number of ELISA or RIA-based systems for detection of TNF are commercially available. Using these assay systems, one has to be aware of the potential effects caused by (1) cross-reactivity with LTα, (2) the presence of TNF inhibitors, and (3) partially degraded biologically inactive TNF.

VI. TNF ANTAGONISTS

The clear indication for TNF involvement in a number of pathophysiological situations has intensified search for potent antagonists of this cytokine suitable to use *in vivo*. Inhibition of TNF action seems feasible at different levels: TNF production and release, cytokine binding to receptors, and receptor signaling.

A. Inhibitors of TNF Production

Although a number of other cytokines have the capability to modulate TNF production in various systems, there is no candidate for a general, potent inhibitor of TNF production. Prostaglandin E2 and glucocorticoids have been found to inhibit TNF synthesis, but both are induced by LPS and TNF themselves, respectively, and might thus be involved in internal feedback loops (reviewed in Beutler and Cerami, 1989). The drug thalidomide has been reported to inhibit TNF synthesis via enhanced degradation of mRNA (Moreira et al., 1993). This mechanism of action is different from that of corticoids or pentoxifylline, both inhibiting mRNA accumulation (Han et al., 1990). In accordance with these data, synergistic action of thalidomide and pentoxifylline has been described (Han et al., 1990). Pentoxifylline strongly down-regulates expression of TNF mRNA but most likely also suppresses expression of other cytokines like IL-2 and IFNγ (Thanhauser et al., 1993).

B. Membrane TNF Proteinase Inhibitors

Major efforts have been undertaken to develop inhibitors of the last step of TNF secretion, that is, the proteolytic cleavage of the membrane bound precursor molecule. Recently described inhibitors of Zn^{2+}-dependent metalloproteinases seem to act in a specific manner (Gearing et al., 1994; McGeehan et al., 1994). Protection against a lethal dose of endotoxin by such inhibitors has been demonstrated in an animal model system using D-galactosamine treated mice (Mohler et al., 1994). Whether application of proteinase inhibitors results in an accumulation of membrane integrated TNF, however, is unclear. In consideration of the recently described segregation of the bioactivities of soluble versus membrane bound TNF (Grell et al., 1995) the biological consequences of such a treatment are hard to predict.

C. TNF-specific Antibodies

A large number of neutralizing TNF-specific monoclonal antibodies have been described. The following characteristics would be expected upon application: Both, the membrane bound form of TNF and the soluble cytokine are recognized and blocked, whereas LTα mediated effects should not be affected. Because of the high affinity of ligand/receptor interaction, reasonably high concentrations of high affinity antibodies are necessary for effective cytokine neutralization. Formation of antibody complexes might result in a reduction of TNF clearance (Kwiatkowski et al., 1993) by similar mechanisms as described in the following section for the soluble receptors. Typically, monoclonal antibodies specific for human TNF are murine proteins, and are thus capable of inducing a neutralizing immune response upon repeated administration in vivo. Antigenicity seems to be highly dependent of the individual antibody and does not necessarily disappear totally upon "humanization" of these proteins. A mouse-human chimeric TNF specific monoclonal antibody (Knight et al., 1993) has been applied in patients with rheumatoid arthritis (see following).

D. Soluble Receptors

The extracellular domains of both receptor molecules have been identified in human urine and serum (Engelmann et al., 1990b; Gatanaga et al., 1990; Lantz et al., 1990; Seckinger et al., 1990). Comparison of the amino- and carboxy-termini revealed that TNF binding protein (TBP) I is not the product of alternative splicing upon transcription but rather reflects a proteolytic cleavage product of TR60. Production of TBP I is inducible, for example, by phorbol ester and is independent on the presence of the intracellular TR60 domain (Brakebusch et al., 1992). Shedding of TR80 in activated human T lymphocytes is also inducible and can be triggered by stimulation of the T cell receptor. Studies with protein kinase inhibitors suggest that the phosphorylation status of the intracellular TR80 domain affects shedding (Crowe et al., 1993). In human neutrophils, TNF itself induces a rapid and selective shedding of TR80 (Porteu and Hieblot, 1994). Although human neutrophil elastase shows a similar specificity for TR80, inhibitory studies suggest involvement of a different enzyme (Porteu et al., 1991). Studies with the histiocytic cell line U937 also showed a differential effect of TNF on both receptors. Whereas TR80 was rapidly shed, internalization of TR60 was induced (Higuchi and Aggarwal, 1994).

In a number of pathophysiological situations, such as systemic lupus erythematosis (Aderka et al., 1993), burns and renal failure (Adolf and Apfler, 1991; Lantz et al., 1991), and rheumatoid arthritis (Dayer, 1991), elevated levels of TNF binding proteins in serum and synovial fluids have been observed. The biological function of these molecules is still a matter of discussion. They have often been considered to represent naturally occurring TNF inhibitors, although some facts might limit their neutralizing capacity.

1. *In vivo*, TNF is very rapidly cleared via the kidney, formation of high molecular weight complexes with the soluble receptors could retain bioactive TNF in the body. In fact, evidence has been presented that TBPs could form a reservoir of bioactive TNF (Aderka et al., 1992).
2. As TBPs are monomers, their affinity for TNF is comparably low. In contrast, trimeric TNF binds two or three membrane receptors, leading to formation of complexes with higher stability. This means that high concentrations of TBPs are necessary to significantly compete with TNF's binding to membrane receptors.

To overcome this problem in a potential clinical application, fusion proteins have been constructed consisting of the Fc part of an immunoglobulin (Ig) connected to the respective extracellular domain of TR60 or TR80. Comparative studies demonstrated that the TNF neutralizing capacity of such a dimeric TR80 derived fusion protein was in fact much higher than that of the respective monomer (Howard et al., 1993; Mohler et al., 1993). Ig fusion proteins have been used to block TNF-stimulated HIV expression in chronically infected cell lines (Howard et al., 1993), to protect mice and primates from septic shock induced by LPS (Mohler et al., 1993; Van Zee et al., 1996), and to reduce the symptoms in type II collagen-induced arthritis in mice (Wooley et al., 1993). An Ig/TR60 fusion protein was also applied to effectively block TNF activity *in vivo* by adenovirus-mediated gene transfer (Kolls et al., 1994). When the TR60 and TR80-derived IgG fusion proteins were directly compared for their efficacy in a septic shock model, the TR60 product showed a much higher protective capacity (Evans et al., 1994). This difference in these recombinant products is presumably a reflection of the unlike association/dissociation kinetics of the two molecules under physiological conditions, but

becomes not apparent when equilibrium dissociation constants are determined at 4 °C (Evans et al., 1994).

E. Receptor-specific Antibodies

TNF receptor-specific antibodies have been used to purify receptor material, to measure TBPs in biological fluids, as well as for agonistic and antagonistic studies *in vitro* and *in vivo*. A number of TR60-specific monoclonal antibodies show agonistic activity, whereas Fab fragments produced thereof have lost this bioactivity (Engelmann et al., 1990a). Similar data have been obtained for TR80-specific antibodies and sera (Tartaglia et al., 1991; Grell et al., 1994), indicating that for both receptors (1) cross-linking is a crucial (and possibly sufficient) event to initiate signaling, and (2) Fab fragments produced from ligand competing antibodies represent valuable and selective receptor antagonists. In a septic shock model in baboons, where TR60 is known to play the predominant role (see the following section), Fab fragments prepared from the TR60-specific mAb H398 did protect the animals (Pfizenmaier et al., unpublished).

F. Interference with Intracellular Signaling

Although our understanding of TNF receptor-induced signal transduction is rapidly growing (see following), there is currently no means by which to selectively interfere with intracellular signal cascades triggered by TNF. This is true because on the one hand the spectrum of TNF-induced signal cascades has not yet been fully elucidated and, on the other hand, several of the known signal pathways are shared with other cytokines. Nevertheless, as selectivity is maintained despite all redundancies, it appears that a certain temporal and spatial pattern of intracellular signals defines signal specificity rather than induction of a single cytokine specific signal itself. Which inhibitor is effective and apparently specific in a given situation might thus depend on the cell type, differentiation state of a given cell, and the conditions of the experiments. Accordingly, a large number of metabolic and signaling inhibitors have been described to block cellular TNF effects. These include inhibitors of phospholipases (Camussi et al., 1990; De Valck et al., 1993), the arachidonate pathway (Suffys et al., 1987; Haliday et al., 1991), and radical oxygen intermediate production (Matthews et al., 1987; Schulze-Osthoff et al., 1992, 1993).

VII. TNF RECEPTORS

Two distinct membrane receptors have been cloned independently by several groups (Himmler et al., 1990; Loetscher et al., 1990; Schall et al., 1990; Smith, C. A., et al., 1990). Both receptors are typical transmembrane proteins with extracellular and intracellular parts of about equal size and a single transmembrane domain. The two TNF receptors are very similar in the size of their protein backbone, the difference in the apparent molecular masses is caused by glycosylation. The gene coding for the smaller receptor protein with an apparent molecular mass of 55-60 kDa (TR60, 455 amino acids) is located on chromosome 12p13 (Baker et al., 1991; Fuchs et al., 1992), that coding for TR80 (70-80 kDa, 461 amino acids) on chromosome 1p36 (Baker et al., 1991). Both are single copy genes. Analyses of the mRNAs suggest near ubiquitous expression of both TNF receptors with a single transcript of 2.3 kb length for TR60 in many tissues, but also alternatively sized transcripts in a few other cell types (Himmler et al., 1990; Schall et al., 1990; Goodwin et al., 1991). The mRNA of the TR80 shows some size heterogeneity with transcripts of 4.5-5 kb, depending on the tissue (Smith, C. A., et al., 1990; Goodwin et al., 1991).

A. The TNF/NGF Receptor Family

Both TNF receptors are members of a recently defined, growing receptor family, the TNF or TNF/NGF receptor superfamily (for review see Beutler and van Huffel, 1994; Smith et al., 1994). This family, interacting with a parallel family of ligands, is defined by a characteristic repeating cysteine-rich motif in the extracellular domains of its members. TR60 and TR80 contain 24 and 22 cysteine residues, respectively, in their extracellular domains. Both receptors form homologous fourfold pseudo-repeats of about 40 amino acids including six cysteines at conserved positions. Up to now, the family is composed of the following members: TR60 and TR80, the APO-1/Fas antigen (Itoh et al., 1991; Oehm et al., 1992), the low affinity p75 NGF receptor, the LTα-binding TNF receptor related protein (Crowe et al., 1994), CD27, CD30, the B cell activation antigen CD40, the rat OX40 antigen, the T cell antigen Ila (4-1BB; Schwarz et al., 1993), and the TRAIL receptor (Wiley et al., 1995).

The product of an open reading frame (T2) in the genome of Shope fibroma virus, a tumorigenic poxvirus inducing malignancies in rabbits, also contains four of the cysteine-rich 40 amino acid repeats homologous

to the ones mentioned previously and is capable of binding TNF (Smith, C. A., et al., 1991). Further, cowpox virus encodes two soluble, secreted forms of TNF receptors. One of these proteins is capable to bind both TNF and LTα, the other is specific for TNF only (Hu et al., 1994).

The homology between TR60 and TR80 is restricted to the extracellular domains only; no evidence for similar signal coupling can be obtained from the sequences of the intracellular domains. In fact, none of the intracellular domains of all defined members of the TNF receptor family gives any indication for the mechanisms of intracellular signaling. Only TR60, the APO-1/Fas antigen and CD40 show some homologies within a short stretch of about 40 amino acids, which is located at different positions of the cytoplasmic domains of the respective molecules, and, in the case of TR60, has been demonstrated to be essential for induction of cytotoxicity (Tartaglia et al., 1993a).

B. Regulation of Receptor Expression

Clearly, expression of both TNF receptors is controlled by distinct mechanisms. In general, expression of TR80 seems to be more strongly affected by the activation status of the cell, whereas TR60 shows a more constitutive expression pattern. In various cell lines from myeloid origin, normally coexpressing about equal numbers of both receptors, a number of studies show inducible expression of TR80: Activators of protein kinase A, like cAMP, strongly up-regulate this molecule, whereas TR60 remains unaffected (Scheurich et al., 1989; Thoma et al., 1990). Protein kinase A-mediated up-regulation of TR80 in U937 cells correlates with a corresponding increase in TNF mRNA and can be induced also by GM-CSF in this cell line (Chambaut-Guerin and Thomopoulos, 1991). Other cytokines, able to up-regulate TR80 in several cell lines, are IFNα, β, and γ (Aggarwal et al., 1985b; Tsujimoto et al., 1986; Billard et al., 1990). In lymphokine-activated killer cells, IL-1β, IL-4, and IL-6 up-regulate TR80 expression (Dett et al., 1991). TNF and IL-1 selectively up-regulate TR80 expression in the fibroblastoid cell line SV-80 at the mRNA level (Winzen et al., 1993). TNF also up-regulates TR80 mRNA in malignant epithelial cell lines, whereas TR60 mRNA remains unaffected (Kalthoff et al., 1993).

Regulation of TR60 has been observed in the course of cellular differentiation: When HL60 differentiates into macrophages, but not granulocytes, TR60 is strongly down-regulated at the levels of mRNA and membrane expression (Winzen et al., 1992). In U937 cells, 1,25-di-

hydroxyvitamin D3 down-regulates TR60 (Chambaut-Guerin and Thomopoulos, 1991). In some cell lines, TNF itself has been found to affect TR60 expression. In the fibroblastoid cell line SV-80 and in human keratinocytes TR60 is down-regulated by TNF (Trefzer et al., 1993; Winzen et al., 1993), whereas in neutrophils (Porteu and Hieblot, 1994) and U937 cells (Higuchi and Aggarwal, 1994) shedding of TR80 is induced by the ligand. Interestingly, TR80 shedding in U937 cells is induced by ligand binding to TR60 (Higuchi and Aggarwal, 1994), an example of heterologous receptor down-regulation.

In a large number of cells tested, activators of protein kinases C, for example, phorbol esters, rapidly induce down-regulation of TNF binding capacity (Aggarwal and Eessalu, 1987; Unglaub et al., 1987). Both receptor types are affected, although with different kinetics. Whereas TR80 binding capacity is down-regulated within minutes (Unglaub et al., 1987), TR60 mediated ligand binding decreases with a half-life time of about 20 minutes (Scheurich, unpublished data). The mechanism of down-regulation includes proteolytic cleavage (Gatanaga et al., 1991; Higuchi and Aggarwal, 1993, 1994), giving rise to the presence of the so-called TNF inhibitors in serum and other body fluids. Induction of receptor shedding was also obtained by treatment of U937 cells with the serine/threonine phosphatase inhibitor okadaic acid (Higuchi and Aggarwal, 1993). In a number of cell lines this down-regulatory effect is overcome by a subsequent up-regulation of TR60 expression, most likely induced at the mRNA level (Chambaut-Guerin and Thomopoulos, 1991; Aggarwal et al., 1993).

Resting T and B lymphocytes are virtually TNF receptor negative (Scheurich et al., 1987; Erikstein et al., 1991). Upon primary activation, expression of both receptors is reversibly induced, but might be regulated independently (Erikstein et al., 1991). Freshly isolated B cells from CLL patients are virtually TNF receptor negative, but readily express receptors upon culture *in vitro* (Digel et al., 1990).

VIII. INTRACELLULAR SIGNALING

The broad spectrum of TNF responses correlates with a wide array of different, but not necessarily independent, intracellular events which can be induced by this cytokine. Besides obvious cell type-specific differences in responsiveness, several possible reasons for this diversity have to be kept in mind. First, the fact that most cells coexpress both receptor

types, although in different proportions, directly points to the question of their respective contribution to a final cellular response. This is emphasized by findings that these receptors are subjected to regulation even by TNF itself (see preceding). Second, response heterogeneity is frequently caused by diversification of post-receptor signal transduction pathways. Third, membrane receptors may not only function as transmembrane signaling-components, but may act as transporters of TNF, which itself, or complexed to its receptor, may exhibit intracellular signaling activity (Smith, M. R., et al., 1990). And finally, the response pattern induced by the receptors might also differ when triggered by either the soluble or the transmembrane form of TNF, or by LTα.

As the cytoplasmic sequences of both receptor molecules show no obvious similarity to any so far described protein kinase nor to any other enzyme, physical interaction with receptor-associated components are thought to activate intracellular enzymatic activities. Indeed, receptor-associated kinases have been described for both receptor types (Darnay et al., 1994a, 1994b; Van Arsdale and Ware, 1994) and, therefore, may represent the immediate connection to a cascade of phosphorylation events elicited by TNF (Guy et al., 1992; Van Lint et al., 1992). Besides this, the molecular cloning of proteins associated with TNF receptors defines additional mechanisms coupling downstream signaling cascades to these receptors. These include the TNF receptor-associated factors (TRAFs), originally cloned as TR80-associated molecules (Rothe et al., 1994) and subsequently shown to be important for TNF-mediated activation of the transcription factor NF-κB (Rothe et al., 1995a). The TRAFs have in common a highly conserved C-terminal domain, the TRAF domain, which mediates specific binding to receptors of the TNF receptor superfamily. Another group of molecules comprises TRADD, FADD (for TNFR- and Fas-associated death domain protein, respectively) and RIP (receptor interacting protein), all implicated in TNF-induced apoptosis (for review see Fraser and Evan, 1996). They have in common a so-called death domain, a module previously described for TR60 and Fas themselves, which is necessary and sufficient for signaling cell death (Hsu et al., 1995, 1996a, 1996b). In addition, TRAFs interact with homologues of the baculovirus-derived inhibitors of apoptosis (IAPs; Rothe et al., 1995b) and further regulatory molecules, termed I-TRAF/TANK and A20 (Rothe et al., 1996; Cheng and Baltimore, 1996; Song et al., 1996).

From the kinetics of signaling events induced by TNF one of the very early intracellular events is the production of diacylglycerol (DAG)

being released from membrane phospholipids by activation of a phosphatidylcholine-specific phospholipase C (PC-PLC) (Schütze et al., 1991). DAG generation seems to be central for the activation of at least two important groups of enzymes in TNF signaling, members of the protein kinase C (PKC) family, and another C type phospholipase, the acidic sphingomyelinase (SMase). Activation of the latter enzyme leads to the cleavage of its substrate to phosphocholine and ceramide. Work of several groups strongly suggest a functional role of ceramide in TNF signal transduction, as exogenously added ceramides, similar to TNF treatment, induce a number of cellular TNF responses (Hannun 1994) like differentiation of HL60 cells, c-myc proto-oncogene down-regulation (Kim et al., 1991), activation of the transcription factor NFκB (Schütze et al., 1992; Yang et al., 1993) or apoptosis (Obeid et al., 1993). The acidic pH optimum of a TNF responsive SMase indicates its location in an endosomal or lysosomal compartment, suggesting that this enzyme is activated during internalization of the TNF/TNF receptor complex. However, another type of SMase with a neutral pH optimum has also been implicated in TNF signaling (Kim et al., 1991). Ceramide produced from membrane sphingomyelin by this enzyme has been shown to activate a protein phosphatase (Dobrowsky and Hannun, 1992) as well as a membrane-bound kinase (Liu et al., 1994). This ceramide-activated protein kinase (CAP) phosphorylates substrates containing the motif X-Ser/Thr-Pro-X and is thereby a member of an emerging family of proline-directed serine/threonine protein kinases, to which the mitogen-activated protein (MAP) kinases belong (Kolesnick and Golde, 1994). Activation of p42/44 MAP kinases by TNF was originally demonstrated in fibroblasts (Van Lint et al., 1992) but was shown later to also be inducible within minutes in HL60 cells by sphingomyelinase and ceramide (Raines et al., 1993). Stimulation of the MAP kinase cascade is a well known feature of numerous ligand/receptor systems which can activate enzymes like PLA_2 or transcription factors like c-*fos* and c-*myc* (Blenis 1993).

The substitution of TNF signaling by ceramide places the activation of the sphingomyelin pathway early in the signaling events elicited by TNF. However, a discrimination between ceramide metabolites produced by either the neutral or the acidic SMase seems to be inevitable. The topology of ceramide generation either at the cell membrane or in acidic compartments appears to be the reason for the differential triggering of the MAP kinase/PLA_2 pathway or the pathway leading to NFκB activation, respectively. Demonstration of such a distinction was brought

about by means of cytoplasmic deletion mutants of TR60, showing that the TR60 regions coupled to neutral or acidic SMase, respectively, are different (Wiegmann et al., 1994).

Currently it is still unclear how the activation of further downstream signal molecules is accomplished. An important role in TNF-induced signaling has been attributed to a recently identified subgroup of MAP kinases, the c-Jun NH_2-terminal kinases (JNKs) which specifically phosphorylate the transcription factor c-*Jun* and, therefore, might play a crucial role in AP-1-stimulated gene expression (Kyriakis et al., 1994). In addition, in several cell lines protein kinase C activation and a rapid translocation from cytosol to the cell membrane was observed coinciding with the occurrence of the typical PKC activator DAG (Schütze et al., 1990, 1991). However, since no concomitant rise in inositol trisphosphate and intracellular calcium was revealed, the role of conventional PKCs in TNF signaling remains obscure. In particular, TNF-induced NFκB activation has been shown to be independent of conventional PKCs (Meichle et al., 1990). Recent work has suggested a role for novel or atypical PKC subtypes in TNF-induced NFκB activation. Accordingly, PKCξ can be directly activated by ceramide and might thus represent the crucial link between the sphingomyelin pathway and activation of NFκB (Diazmeco et al., 1993; Müller et al., 1995).

The pro-inflammatory cellular response to TNF is probably associated with the TNF-mediated stimulation of PLA_2 and the subsequent release of arachidonic acid. This will lead to the production of inflammatory lipid mediators like prostaglandins and leukotrienes (Lin, et al., 1993; Ueno et al., 1990; Krönke et al., 1992). Further, a role of PLA_2 has also been also proposed for the TNF-mediated induction of cytotoxicity as inhibitors of the arachidonate metabolism like the corticosteroid dexamethasone, reduce TNF cytotoxicity (Suffys et al., 1987). Moreover, the introduction of PLA_2 activity into otherwise enzyme-deficient cells restores their sensitivity toward TNF (Hayakawa et al., 1993). The activation of PLA_2 might occur by p42 MAP kinase-mediated phosphorylation (Lin et al., 1993, Nemenoff et al., 1993). As the stimulation of neutral SMase by TNF can activate the MAP kinase pathway (see preceding) it is tempting to speculate that this might be the link between TNF receptor activation and the arachidonate metabolism. Finally, arachidonate intermediates may then affect expression of TNF sensitive genes as shown for the TNF induction of c-*fos*, which is dependent on the arachidonic acid-lipoxygenase pathway (Haliday et al., 1991). The generation of eicosanoids by the different lipoxygenases and

a subsequent production of reactive free radicals in the mitochondria have been discussed to be cofactors for TNF-induced gene induction as for TNF-mediated cytotoxicity (Yamauchi et al., 1989; Schulze-Osthoff et al., 1992; Heller and Krönke, 1994). However, it should be stressed here that many of the cytoplasmic mechanisms in TNF signaling elucidated in the last years seem to be more or less cell type-specific and other enzymes, such as, for example, phospholipase D, protein kinase A, tyrosine specific protein kinases, or G-proteins might play an essential role in certain types of tissues (also reviewed in Pfizenmaier et al., 1992; Heller and Krönke, 1994; Beyaert and Fiers, 1994).

TNF-activated intracellular signaling finally results in the activation of a multitude of different genes conferring many of the pleiotropic activities of this cytokine. Among them are genes coding for other cytokines such as IL-1, IL-6, and TNF itself, receptors such as the IL-2 receptor α chain, adhesion molecules, acute phase proteins, and other inflammatory mediators like tissue factor, collagenase, and stromelysin. For a detailed overview of TNF-inducible transcription factors and TNF-responsive genes see Krönke et al. (1992). It is noteworthy that both transcriptional and translational events appear to be important in determining the level of induced gene expression. Further, many of the genes regulated by TNF are also controlled by other cytokines, in particular IL-1. These similarities in gene induction between different cytokines is most likely brought about by shared intracellular signaling mechanisms as, for example, both TNF and IL-1 are potent inducers of the transcription factor NFκB (Osborn et al., 1989a). Although this might explain the wide-spread overlap in cytokine effects, it renders analysis of the *in vivo* role of the particular cytokines more difficult.

IX. FUNCTIONAL ROLE OF RECEPTORS

One point of great interest during the past few years focused on the question of the functional role of the two TNF receptors. In accordance with mRNA expression, binding studies with iodinated ligand have revealed that most cell lines and tissues coexpress both receptor molecules (Brockhaus et al., 1990; Shalaby et al., 1990; Thoma et al., 1990; Scheurich et al., 1993). This argues against a strict tissue-specific function of TR60 and TR80, although the ratio of expression varies strongly. Typically, epithelial cells express predominantly TR60 and lymphoid cells mainly TR80 (Hohmann et al., 1989). However, it has been dem-

onstrated that a high receptor number of a given cell type does not necessarily indicate principal cellular responsiveness (Ücer et al., 1987), nor does the numerical prevalence of one receptor type coincide with activation of a typical signal pathway (Grell et al., 1993). Accordingly, neither from the type nor the quantity of expressed membrane receptors can the quality and strength of a cellular response be predicted.

Therefore, the role of each of the two TNF receptor types in certain TNF responses has been inferred from different approaches: (1) Using agonistic and antagonistic receptor-specific antibodies, the participation of the respective receptor type in many TNF-specific responses have been elucidated (Scheurich et al., 1993). It has been shown that most of well-known TNF responses like induction of cytotoxicity (Engelmann et al., 1990a; Shalaby et al., 1990; Thoma et al., 1990), induction of genes, for example, for IL-6, MnSOD or plasminogen activator inhibitor (Tartaglia et al., 1991), but also stimulation of proliferation in fibroblasts (Austgulen et al., 1987; Hori et al., 1988) or enhancement of an antiviral state (Wong et al., 1992) can be attributed to TR60. (2) The fact that human TNF binds only to the murine type I (TR60 homologue) but not to the type II receptor (TR80 homologue) has also demonstrated the principal capability of TR60 to trigger a wide array of bioresponses on its own (Lewis et al., 1991). (3) The generation of mutants of the TNF molecule (muteins), which have been demonstrated to interact almost selectively with only one of the two TNF receptors, thereby representing efficient receptor-specific tools has confirmed the above mentioned findings (Loetscher et al., 1993; Van Ostade et al., 1994). In addition, an important role for TR80 in the formation of severe systemic side effects of TNF has been demonstrated by the diminution of such effects using a TR60 specific mutein in an *in vivo* mouse tumor model (Van Ostade et al., 1993). (4) Finally, a crucial role of TR60 in the onset of septic shock syndrome has been demonstrated by the resistance against LPS and *S. aureus* enterotoxin B-induced shock of homozygous TR60 deletion mutant mice (Pfeffer et al., 1993; Rothe et al., 1993).

Up to now, the vast majority of intracellular signaling events (see previously) has also been associated with signaling via TR60. Transfection of a cDNA construct of the human TR60 into the mouse pre-B cell line 70Z/3, which only expresses murine TR80 and does not respond to mouse or human TNF in regard to activation of the nuclear factor NFκB, phosphatidyl choline specific phospholipase C, phospholipase A_2, or MAP kinase rendered the transfectants responsive for both mouse and human TNF in all of these assay systems (Wiegmann et al., 1992).

On the other hand, the functional role of TR80 is only beginning to be understood. Earlier studies had already suggested that TR80 plays a role in TNF-induced proliferation of thymocytes (Ranges et al., 1988) and activated T lymphocytes (Gehr et al., 1992; Tartaglia et al., 1993b). Studies with receptor-specific agonistic antibodies have now demonstrated that TR80 is by itself able to trigger apoptosis as well (Heller et al., 1992; Grell et al., 1994). Besides a role for TR80 as an independent signaling molecule, a supportive role for TR80 in TR60-mediated responses has been postulated: The rapid TNF/TR80 association and dissociation kinetics were taken as a basis to propose the model of "ligand passing," that is, presentation of TNF to TR60 by TR80 resulting in an increased local TNF concentration at the cell surface (Tartaglia et al., 1993c). Second, cooperation at an intracellular level can be deduced from several studies employing costimulation of receptors with receptor specific antibodies (Gehr et al., 1992; Abe et al., 1993; Bigda et al., 1994). In support of this, homozygous deletion of the TR80 gene in mice shows a reduced sensitivity in high dose LPS mediated shock syndrome (Erickson et al., 1994) which has previously been shown to be TR60 dependent (Pfeffer et al., 1993; Rothe et al., 1993).

X. CELLULAR RESPONSES

A. Necrosis/Apoptosis

One of the most extensively studied cellular effects of TNF is its capability to induce cytotoxicity in some cells. Typically, normal cells are TNF-resistant or may be even stimulated in proliferation, as demonstrated for lymphoid cells and diploid fibroblasts (Kohno et al., 1990; Gehr et al., 1992; Tartaglia et al., 1993b), whereas a number of tumor cells display high TNF sensitivity (Sugarman et al., 1985). This at least partial selectivity has received great attention during the past few years, although TNF exerts its cytotoxic effects on most tumor cells only when protein synthesis is blocked. It has therefore been suggested that TNF—and in a similar way IL-1—induce protein synthesis-dependent protective mechanisms against its own cytotoxicity (Wallach et al., 1988). A number of proteins synthesized upon TNF treatment have been described (Beresini et al., 1988; Lee, T. H., et al., 1990, 1992; Lee, G. W., et al., 1993; Wisniewski et al., 1993), but their role in the modulation of TNF responsiveness remains to be elucidated. The finding that TNF

exerts cytotoxic effects also for virus infected cells (Duerksen-Hughes et al., 1989; Ohno et al., 1993) might indicate a potential role of TNF *in vivo.* Accordingly, cells could be more effectively killed by TNF (in cooperation with other cytokines) when their protein synthesis is inhibited by viral infection.

TNF is able to induce apoptosis similar to the related protein APO-1/Fas ligand (for review see Nagata, 1994). Typical indications as membrane blebbing, condensation of chromatin, and DNA fragmentation have been demonstrated for a number of different TNF treated cell types (Laster et al., 1988; Wright et al., 1992; Woods and Chapes, 1993; Grell et al., 1994). Importantly, there are examples for TNF's capability to induce either apoptosis or necrosis, dependent on the cell type (Laster et al., 1988). There is good evidence for the involvement of membrane-bound TNF in triggering of cytotoxicity during cell-cell contact. Whereas CD8 positive T lymphocytes might mainly kill by perforin and not by membrane bound TNF, as indicated by the fast kinetics of lytic action (Ratner and Clark, 1993), an important role for membrane TNF in cell killing by macrophages (Fishman, 1991; Peck et al., 1991; Sypek and Wyler, 1991; Sypek et al., 1993) and the cytotoxicity of CD4 positive T lymphocytes (Tite, 1990; Liu et al., 1992; Smyth and Ortaldo, 1993) has been suggested.

A large number of chemical compounds, cytokines, and other effectors have been described to modulate TNF's capability to induce cytotoxicity. Induction of TNF resistance could be achieved at different levels, for example, expression of functional receptors, receptor signaling, induction of protective effectors, and finally inhibition of intracellular effector mechanisms. As outlined above, no generally applicable mediators are known, able to down-regulate TNF receptor expression, thus inducing TNF unresponsiveness. Induction of receptor shedding by activation of protein kinase C might represent one of the few mechanisms to reversibly induce TNF desensitization (Unglaub et al., 1987), although numerous additional intracellular effects are triggered. As recently summarized by Beyaert and Fiers (1994), upon induction of apoptosis/necrosis by TNF, stimulation of at least four different phospholipases might be involved in a cell specific manner. Accordingly, various inhibitors of phospholipase A_2 and phospholipase C show some protective activity, indicating critical involvement of these signaling ways in at least some cell types. Various proteinase inhibitors do also block cytotoxic TNF effects. Molecules acting at different levels were effective, like alkylating serine proteinase inhibitors or proteinase substrates (Ruggiero et al.,

1987; Suffys et al., 1988). Very recent data have demonstrated coupling of a cystein protease related to the interleukin-1β-converting enzyme (ICE) to TR60 and Fas, termed MACH (Boldin et al., 1996) or FLICE (Musio et al., 1996).

There is good evidence for the involvement of oxidative damage in TNF-induced cytotoxic mechanisms. Inhibitors of reactive oxygen intermediates (ROI) show protective activity (Lancaster et al., 1989; Schulze-Osthoff et al., 1992; Grell et al., 1994) and there is a good correlation between the intracellular glutathione level and TNF resistance (Matthews et al., 1987; Yamauchi et al., 1989). Recently, L929 cell clones devoid of functional mitochondria have been obtained by prolonged treatment with ethidium bromide and chloramphenicol. These cells lacked virtually any TNF sensitivity (Schulze-Osthoff et al., 1993). In parallel to the induction of ROI, TNF induces the accumulation of manganous superoxide dismutase (MnSOD; Wong and Goeddel, 1988). Expression of antisense MnSOD mRNA renders some cells highly TNF sensitive whereas overexpression of MnSOD induces TNF resistance (Wong et al., 1989). These data indicate a critical role for MnSOD in TNF-induced protection against its cytotoxic effects in some cells, but not in all (Powell et al., 1990). Additional antioxidant enzymes like the CuZnSOD, catalase, glutathione peroxidase, or transferase are not induced by TNF.

Another protein with a potential protective role is the major heat-shock protein hsp70. Overexpression of hsp70 renders WEHI-S cells partly resistant against monocyte mediated cytotoxicity, whereas expression of hsp70 antisense mRNA enhances sensitivity (Jäättelä and Wissing, 1993). In this model, signaling via activation of PLA_2 might be important, as hsp70 overexpression inhibits TNF induction of this enzyme (Jäättelä, 1993).

B. Other Cellular Effects

Countless additional TNF effects have been described besides the induction of cytotoxicity. Clearly, TNF possesses the capability to modify the program of gene expression of its target cells in a cell type-dependent manner and thus affects many genes at the level of transcription (reviewed in Krönke et al., 1992; Jäättelä, 1991). Crucial for the immunoregulatory activity of TNF is its ability to control the expression of a number of cell surface antigens, such as HLA class I and class II antigens (Johnson and Pober, 1990; Pfizenmaier et al., 1987), ICAM (Haskard et al., 1986), E-selectin (ELAM; Bevilacqua et al., 1987, 1989),

and VCAM (Osborn et al., 1989b), a number of cytokines such as IL-6 (Zhang et al., 1990), IL-1 (Nawroth et al., 1986; Dinarello et al., 1986), GM-CSF (Munker et al., 1986), and TNF itself (Hensel et al., 1989), as well as receptors such as those for IL-2 (Scheurich et al., 1987; Lee, J. C., et al., 1987) and EGF (Palombella et al., 1987). Generally, TNF seems to be a costimulator for cells of the immune system, such as T and B lymphocytes (Scheurich et al., 1987; Lee, J. C., et al., 1987; Jelinek and Lipsky, 1987; Hackett et al., 1988; Yokota et al., 1988; Boussiotis et al., 1994), monocytes and macrophages (Philip and Epstein, 1986; Camussi et al., 1987), and neutrophils (Klebanoff et al., 1986; Atkinson et al., 1988). Of great importance might be the effects of TNF on osteoblasts and osteoclasts, leading to reduced bone synthesis and elevated bone resorption, respectively (for review see Mundy, 1989; Mundy et al., 1992). Further, the stimulatory action of TNF on chondrocytes leading to increased synthesis of collagenase, gelatinase, and stromelysin is believed to play an important role in chronical inflammatory diseases (see following).

XI. TNF *IN VIVO*

There is a clear discrepancy between the enormous number of publications describing *in vitro* effects of TNF and our understanding of this cytokine's physiological role. The main reasons for this are related to the pleiotropic activity of TNF combined with the potential TNF responsiveness of cells from virtually all tissues. The following *in vivo* aspects of TNF have been of great interest: immunomodulatory/proinflammatory effects, septic shock, and cachexia.

A. Immunomodulation

TNF possesses an immunostimulatory potential, believed to be important in inflammatory immune responses. Neutrophils are capable of responding to TNF within minutes showing enhanced adherence to endothelial cells (Gamble et al., 1985). In addition, TNF primes neutrophils toward a higher responsiveness to a subsequent challenge from agents like unopsonized zymosan or the chemotactic peptide formyl-Met-Leu-Phe (Klebanoff et al., 1986; Atkinson et al., 1988). Admission of TNF into mice results in a neutrophil release from the bone marrow (Remick et al., 1987). Endothelial cells are converted by TNF from an

anticoagulant into a procoagulant state via increased synthesis of plasminogen activator inhibitor and suppression of thrombomodulin and plasminogen activator itself (for an overview see Clauss et al., 1992; Gamble et al., 1992). In addition, expression of metalloproteinases is stimulated (Hanemaaijer et al., 1993). The main producer cells of TNF, monocytes and macrophages, are stimulated by TNF treatment to exert enhanced cytotoxicity and production of IL-1, prostaglandin E_2, and platelet-activating factor (Philip and Epstein, 1986; Bachwich et al., 1986; Camussi et al., 1987). TNF indirectly affects expression of additional cytokines such as IL-6 and IFNβ (derived from fibroblasts), IL-8 (many cell types), and GM-CSF (fibroblasts, endothelium). Accordingly, TNF participates in a multifactorial concert leading to development of an inflammatory host response (for reviews see Beutler and Cerami, 1989; Tracey and Cerami, 1993).

Less data are available regarding the *in vivo* effects of TNF on the specific immune system. B cell responses can be stimulated by TNF, as the antibody response directed against T cell dependent antigens is enhanced, but not that triggered by a T cell independent antigen (Ghiara et al., 1987). *In vivo* activated mature B cells isolated from human tonsils are dependent on exogenously added TNF to secrete high levels of IgG (Rodriguez et al., 1993). Evidence for *in vivo* stimulation of T cell-mediated cytotoxicity comes from experiments in mice where TNF stimulates development of a specific secondary immune response to a transplanted tumor upon tumor rejection (Palladino et al., 1987).

B. Septic Shock

Clinical septic shock is still associated with a high mortality rate and is typically caused by endotoxins from gram negative bacteria. But also exotoxins such as *Escherichia coli* hemolysin, gram positive *Staphylococcus aureus* α toxin, or *S. aureus* enterotoxins can induce the cascade leading to shock syndrome. The septic shock syndrome is associated with fever, hypotension, oliguria, metabolic acidosis, development of hemorrhagic lesions, and other symptoms (for reviews see Tracey, 1991; Tracey and Cerami, 1993). It is generally accepted that in gram negative sepsis LPS initiates the release of a number of soluble mediators, of which TNF plays a key role. This hypothesis is substantiated by the finding that (1) LPS treatment induces TNF release into the serum of experimental animals (Tracey et al., 1986) and humans (Michie et al., 1988), (2) TNF application induces a shock syndrome hardly distin-

guishable from that triggered by bacteria (Tracey et al., 1986), and (3) blocking of TNF or TNF signaling significantly reduces the lethality of endotoxin (Beutler et al., 1985b) and *E. coli* (Tracey et al., 1987).

Studies in humans with meningococcal disease caused by the endotoxin producer *Neisseria meningitides* have revealed a clear correlation between TNF serum levels and severity of the disease. All patients with a serum concentration > 0.1 ng/ml died (Waage et al., 1987). There is good evidence that TNF and endotoxin act synergistically, that is, TNF is less toxic in the absence of LPS (Rothstein and Schreiber, 1988). One key mediator involved in this synergism is IL-1, which strongly potentiates TNF effects in mice, but is hardly toxic by itself (Waage and Espevik, 1988). Cooperative action of TNF with IFNγ and GM-CSF has been also suggested from animal models (Talmadge, 1992; Tiegs et al., 1994). It has also been suggested that interleukins 6 and 8 play a role in the development of shock (Waage et al., 1989; Chollet-Martin et al., 1993; Dinarello et al., 1993), although recent data show that IL-6 deficient mice, untreated or galactosamine sensitized, show no significant protection toward murine TNF (Libert et al., 1994).

C. Cachexia

One of the original definitions of TNF is based on its extended metabolic effects when systemically present for prolonged times. Chronic trypanosome infection in rabbits served as an animal model to study TNF-mediated cachexia associated with triglyceridemia (Beutler et al., 1985a). Only low numbers of parasites were sufficient to induce, via prolonged release of sublethal doses of TNF, typical symptoms like anorexia, weight loss, and depletion of lipid and protein resources, finally leading to death (Rouzer and Cerami, 1980). *In vitro* studies with adipocytes have demonstrated an inhibitory effect for TNF on several enzymes of the lipogenic pathway, especially lipoprotein lipase downregulation at the level of transcription (Cornelius et al., 1988; Zechner et al., 1988). In addition, catabolic pathways of lipid degradation and glycogenolysis in muscle cells are stimulated (Lee, M. D., et al., 1987).

D. TNF in Disease

Bacterial and Parasitic Infections

A number of animal infection models indicate a major role for TNF in host defence against facultative intracellular bacteria and parasites. In

a murine model of listeriosis, early administration of TNF after inoculation of a lethal dose of *Listeria monocytogenes* protects the animals from death (Desiderio et al., 1989). Neutralizing TNF-specific antibodies, on the other hand, delay the elimination of bacteria when these had been given at a sublethal dose (Nakane et al., 1988; Havell, 1989). Besides TNF, raised levels of IFNγ and IL-6 can also be detected in the animals. These cytokines are believed to cooperate during elimination of bacteria (Nakane et al., 1992). IFNγ might play a key role for induction of TNF production (Nakane et al., 1992; Havell, 1993; Langermans et al., 1992). A similar pattern of cytokine cooperation arose from models where mice were infected with *Legionella pneumophilia,* a gram negative bacterium which has been defined as the etiologic agent of legionnaires' disease. TNF is produced in response to infection (Arata et al., 1993) and has been shown to cooperate with IFNγ in defence, most likely dominated by polymorphonuclear cells (Blanchard et al., 1988, 1989). Animal infections with mycobacteria, leading to the clinical symptoms of leprosy and tuberculosis, represent other biological systems where crucial involvement of TNF was demonstrated. Several components of the bacterial cell wall have been shown to stimulate peripheral blood mononuclear cells and to induce TNF production in macrophages *in vitro* (Valone et al., 1988; Bradbury and Moreno, 1993; Ab et al., 1990). Peripheral blood mononuclear cells derived from tuberculosis patients were shown to constitutively produce enhanced amounts of TNF compared to normal controls (Cadranel et al., 1990). Serum TNF titer may be enhanced in patients with leprosy (Pisa et al., 1990), but not in patients with tuberculosis (Rook et al., 1989). There is good evidence that TNF, in cooperation with IFNγ, IL-2, and additional cytokines, enhances killing of mycobacteria by macrophages *in vitro* (Bermudez and Young, 1988; Bermudez et al., 1990). Clearly, TNF is associated with formation of granuloma, representing organized accumulations of macrophages and lymphocytes, thus enabling the disposal of pathogens in the host. Granuloma formation in the liver of BCG treated mice correlates with the TNF level determined (Kindler et al., 1989) and, in fact, the inability of SCID mice to form granuloma after infection with schistosoma can be restored by exogenous TNF (Amiri et al., 1992).

In malaria disease, it was early shown that macrophages are primed for TNF release (Wood and Clark, 1984). Indeed, the side effects observed in clinical TNF trials in cancer patients were very similar to that observed in *P. falciparum* malaria patients. Mice at an early stage of a *plasmodium vinckei* infection show high sensitivity toward an appli-

cation of exogenous TNF and develop symptoms typically for a terminal stage of this disease (Clark et al., 1987). A good correlation between endogenous TNF (and IL-6) levels and the severity of the disease was found in a number of studies in man (Grau et al., 1989a; Kern et al., 1989; Butcher et al., 1990). These data all indicate that some of the typical clinical malaria symptoms are caused by TNF and not by the parasite itself. On the other hand, there are a number of data suggesting a protective role of this cytokine. In a simian model of malaria, it was shown that TNF kills intraerythrocytic gametocytes of *P. cynomolgi* (Naotunne et al., 1991). In a murine system with *P. yoelii*, evidence was obtained that TNF inhibits the hepatic stages of malaria because of the induction of IL-6 (Nussler et al., 1991).

A different picture has evolved in cerebral malaria, a rapidly progressive encephalopathy with up to 50% mortality. Data from murine models strongly suggest that cerebral pathology is a direct consequence of high levels of TNF. When mice are injected with neutralizing TNF specific antibody days after parasite inoculation, the animals are fully protected against cerebral malaria without change in parasitemia (Grau et al., 1987). Preliminary data from a recent study with Gambian children showed a dose dependent decrease in fever upon treatment with anti TNF antibodies, although the fatality rate seemed not to be affected (Kwiatkowski et al., 1993). IFNγ might play a role in both enhancement of TNF production and in TNF action, as a neutralizing IFNγ-specific monoclonal antibody prevents development of cerebral lesions (Grau et al., 1989b). For the human population as a whole, however, the benefits of the TNF response to a malaria infection might outweigh its disadvantages by far (Kwiatkowski, 1993).

Viral Infections

Evidence for the involvement of TNF in viral infections comes from a number of observations. DNA and RNA viruses induce expression of TNF upon infection (Aderka et al., 1986). TNF possesses antiviral enhancing activity in the presence of IFNγ (Wong et al., 1992). Peripheral blood mononuclear cells isolated from patients after *in vivo* application of TNF show a strong protection against infection with vesicular stomatitis virus (Nokta et al., 1991). However, in some cells (Mestan et al., 1988: Hughes et al., 1988; Chen et al., 1993), but not in all (Gessani et al., 1988), the anti-viral activity of TNF might be mediated indirectly by induction of IFNβ. Virus infected cells display increased sensitivity

toward the cytotoxic activity by TNF, for example, produced by natural killer cells (Duerksen-Hughes et al., 1989; Wong et al., 1992). In HIV infection, TNF might play a dual role. On the one hand, treatment of cells with TNF and IFNγ greatly reduces their susceptibility for HIV infection and reduces production of mRNA (Wong et al., 1988). On the other hand, TNF, similarly to IL-1, IL-6, and GM-CSF, markedly stimulates HIV replication in a number of cellular systems (Israel et al., 1989; Matsuyama et al., 1989; Michihiko et al., 1989; Poli et al., 1990). Infection with cytomegalo virus might thus enhance HIV production via stimulation of TNF secretion (Peterson et al., 1992).

Multiple Sclerosis

Pathologically, the brains of multiple sclerosis (MS) patients are characterized by focal perivascular lymphocytic infiltrates and macrophages, areas of demyelination, disappearance of oligodendroglial cells, and proliferation of astrocytes. Mainly T lymphocytes, especially Th1 helper cells (Voskuhl et al., 1993), and macrophages seem to be involved. This pathological picture is very similar to that of experimental autoimmune encephalitis (EAE) in mice, induced by active immunization with myelin basic protein (Paterson, 1977). Although TNF level in the cerebrospinal fluid (CSF) of MS patients may be enhanced only moderately and could be only determined with sensitive RIA techniques (Hauser et al., 1991), but not with conventional ELISA kits (Franciotta et al., 1989; Maimone et al., 1991), a pivotal role of TNF in the pathogenesis of this disease is suggested. It has been shown that T cell clones (Benvenuto et al., 1991) and macrophages (Merrill et al., 1989) derived from CSF of MS patients show enhanced TNF production upon appropriate stimulation. Further, TNF displays a direct cytotoxic activity for cultured mouse spinal cord tissue and rat astrocytes (Robbins et al., 1987; Selmaj and Raine, 1988). When myelin basic protein-specific T cell clones were used to induce EAE in mice, TNF production of these clones correlated well with their potency to induce paralysis in the animals (Powell et al., 1990) and blocking of TNF (and LTα) by monoclonal antibodies prevents transfer of the disease (Ruddle et al., 1990). A central role for TNF, locally limited to the central nervous system, was confirmed by the findings that intracranial injection of neutralizing antibodies and Ig/TNF receptor fusion proteins, respectively, was superior to systemic application (Baker et al., 1994). Transforming growth factor beta (TGFβ) seems to be an antagonist of TNF in EAE (Santambrogio et al., 1993).

Rheumatoid Arthritis

A large number of publications demonstrate the production of TNF by cultured mononuclear cells from rheumatoid arthritis joints (for review see Feldmann et al., 1992; Kollias, 1993; Maini et al., 1993). In addition, immunohistochemical studies have shown the presence of TNF in the synovium and in the cells in the cartilage pannus junction (Chu et al., 1991). In primary and passaged fibroblasts from rheumatoid pannus TNF was shown to induce expression of stromelysin and collagenase in synergism with IL-1 (MacNaul et al., 1990). When synovial cells were cultured *in vitro*, neutralizing TNF-specific antibodies were able to down-regulate IL-1 production (Brennan et al., 1989). Further, in the murine model system of collagen-induced arthritis, the disease enhancing effect of TNF (Brahn et al., 1992) and the beneficial effect of TNF neutralizing antibodies (Piguet et al., 1992; Williams et al., 1994) were shown. Transgenic mice, expressing human TNF under control of modified 3' regulatory sites, develop a chronic inflammatory polyarthritis (Keffer et al., 1991). All these data indicated a pathogenic role of TNF in rheumatoid arthritis and have lead to clinical trials with anti TNF antibodies. Recent data obtained with a chimeric (humanized) antibody show remarkable beneficial effects for the patients treated. Treatment was well tolerated and significant improvements were seen in the Ritchie Articular Index, in serum C-reactive protein levels as well as serum amyloid A and IL-6 (Elliott et al., 1993, 1994a). Quite surprisingly, single anti TNF treatments resulted in long lasting effects for weeks, and treatment cycles could be successfully repeated at least four times (Elliott et al., 1994b).

Cancer

TNF is cytotoxic for some tumor cell lines, but most of them are hardly affected in growth or are even stimulated (see previously). It is therefore unlikely that the anti-tumoral effects of TNF in some animal models (Creasey et al., 1986; Balkwill et al., 1986; Gresser et al., 1986) are due to direct action of the cytokine on tumor cells. In several studies it has been shown that host mediated mechanisms are involved in TNF-triggered tumor regression (Palladino et al., 1987; Manda et al., 1987). More recent data indicate that hemorrhagic necrosis of tumors by TNF is initiated at the endothelial cell level of the intratumoral vessels (Havell et al., 1988; Fahey et al., 1990). The molecular mechanisms of the

apparent selectivity of TNF action on tumor cell vasculature is still undefined and warrants further investigation. In several mouse models, TNF resistant tumor cells were used which had been transfected with TNF to study the effects of local cytokine production (Teng et al., 1991; Blankenstein et al., 1991; Asher et al., 1991). In comparison to control cells, tumor development of TNF producers was markedly inhibited. In one case, tumor tissue showed massive infiltration by macrophages. In T cell deficient nude mice the TNF-mediated tumor growth inhibition was reduced. Interestingly, in these models TNF production was obviously quite low as the animals did not develop cachexia.

Other models, however, revealed a tumor-promoting action of TNF: In an experimental fibrosarcoma metastasis model a single application of TNF was shown to enhance lung metastasis formation after a subsequent challenge with a methylcholanthrene-induced fibrosarcoma line. The augmented proliferative capacity mediated by TNF was suggested to be host-induced and not by direct interaction of TNF with sarcoma cells (Orosz et al., 1993).

The results of numerous clinical TNF studies in cancer patients were, by and large, disappointing (reviewed by Haranaka, 1988; Frei and Spriggs, 1989). Generally, the antitumoral effects were poor, TNF dosage was limited by considerable side effects. Only recently has some progress been achieved. In patients suffering from melanomas or sarcomas of the extremities, dramatic beneficial effects could be obtained by isolated perfusion technique. Extreme dosages of TNF up to 4 mg were used in combination with cytostatics or IFNγ (Lienard et al., 1992a, 1992b; Fahey et al., 1990). Local responses include acute softening and redness of the tumor associated with a strong inflammatory response, similar to TNF mediated anti-tumoral effects in murine systems. These results demonstrate that TNF in combination with additional therapeutic agents (Lejeune et al., 1994) can be clinically very effective in the treatment of some tumors, provided systemic toxicity can be controlled.

XII. SUMMARY

Tumor necrosis factor (TNF) is a typical member of the polypeptide mediator family consisting of soluble factors like the interferons, interleukins, and hematopoietic growth factors. Although the main producer cells of TNF are monocytes/macrophages and lymphocytes, many other cells of the body are capable of producing TNF. Consistent with its

pleiotropic cellular interactions virtually all cells and tissues are potential targets of TNF action.

The control of cellular TNF responsiveness is of extraordinary complexity. First, TNF production is tightly regulated and gives rise to two distinct forms of the bioactive molecule. The active soluble cytokine is proteolytically derived from a membrane-integrated precursor molecule which itself is able to signal in a juxtacrine situation. All typical cellular TNF responses are initiated by specific binding to two homologous receptors which are members of the growing TNF receptor superfamily. These receptors also bind lymphotoxin α and are coexpressed in most cells, although in different proportions. Upon ligand binding, they activate a complex pattern of intracellular signals in a cooperative mode. Both receptors are subject to multiple regulatory mechanisms. Upon stimulation, the extracellular domains can be cleaved proteolytically, giving rise to molecules with inhibitor/storage properties. Besides signaling via receptors, TNF has a lectin-like sugar binding activity, the biological role of which is far from being understood.

Our present understanding of the biological role of TNF comes from both studies defining the conditions of TNF gene expression and investigations unraveling the multiple facets of TNF action. An enormous number of publications has enlightened cellular TNF effects *in vitro* demonstrating a striking diversity. This multitude of phenotypic and functional changes inducible by TNF must depend on a diversification of intracellular signaling mechanisms. However, the biological role of TNF *in vivo* is just beginning to be understood. Obviously, TNF-mediated effects show two sides. On the one hand, evidence is growing that TNF is involved in a number of pathophysiological processes, for example, rheumatoid arthritis, cerebral malaria, and AIDS. On the other hand, TNF is believed to be crucial in the successful development of antibacterial immune responses.

REFERENCES

Ab, B. K., Kiessling, R., Van Embden, J. D., Thole, J. E., Kumararatne, D. S., Pisa, P., Wondimu, A., & Ottenhoff, T. H. (1990). Induction of antigen-specific $CD4^+$ HLA-DR-restricted cytotoxic T lymphocytes as well as nonspecific nonrestricted killer cells by the recombinant mycobacterial 65-kDa heat-shock protein. Eur. J. Immunol. 20, 369-377.

Abe, Y., Gatanaga, M., Osuka, Y., Kimura, S., Burger, R. A., Granger, G. A., & Gatanaga, T. (1993). Role of 55- and 75-kDa tumor necrosis factor membrane

receptors in the regulation of intercellular adhesion molecules-1 expression by HL-60 human promyelocytic leukemia cells *in vitro*. J. Immunol. 150, 5070-5079.

Aderka, D., Holtmann, H., Toker, L., Hahn, T., & Wallach, D. (1986). Tumor necrosis factor induction by *Sendai* virus. J. Immunol. 136, 2938-2942.

Aderka, D., Engelmann, H., Maor, Y., Brakebusch, C., & Wallach, D. (1992). Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J. Exp. Med. 175, 323-329.

Aderka, D., Wysenbeek, A., Engelmann, H., Cope, A. P., Brennan, F., Molad, Y., Hornik, V., Levo, Y., Maini, R. N., & Feldmann, M. (1993). Correlation between serum levels of soluble tumor necrosis factor receptor and disease acitivity in systemic lupus erythematosus. Arthritis Rheum. 36, 1111-1120.

Adolf, G. R., & Apfler, I. (1991). A monoclonal antibody-based enzyme immunoassay for quantitation of human tumor necrosis factor binding protein I, a soluble fragment of the 60 kDa TNF receptor, in biological fluids. J. Immunol. Meth. 143, 127-136.

Aggarwal, B. B., & Eessalu, T. E. (1987). Effect of phorbol esters on down-regulation and redistribution of cell surface receptors for tumor necrosis factor-alpha. J. Biol. Chem. 262, 16450-16455.

Aggarwal, B. B., Kohr, W. J., Hass, P. E., Moffat, B., Spencer, S. A., Henzel, W. J., Bringman, T. S., Nedwin, G. E., Goeddel, D. V., & Harkins, R. N. (1985a). Human tumor necrosis factor. Production, purification, and characterization. J. Biol. Chem. 260, 2345-2354.

Aggarwal, B. B., Eessalu, T., & Hass, P. E. (1985b). Characterization of receptors for human tumor necrosis factor and their regulation by gamma-interferon. Nature 318, 665-667.

Aggarwal, B. B., Graff, K., Samal, B., Higuchi, M., & Liao, W. S. (1993). Regulation of two forms of the TNF receptors by phorbol ester and dibutyryl cyclic adenosine 3',5'-monophosphate in human histiocytic lymphoma cell line U-937. Lymphokine Cytokine Res. 12, 149-158.

Alzani, R., Corti, A., Grazioli, L., Cozzi, E., Ghezzi, P., & Marcucci, F. (1993). Suramin induces deoligomerization of human tumor necrosis factor alpha. J. Biol. Chem. 268, 12526-12529.

Amiri, P., Locksley, R. M., Parslow, T. G., Sadick, M., Rector, E., Ritter, D., & McKerrow, J. H. (1992). Tumour necrosis factor alpha restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. Nature 356, 604-607.

Andersson, U., Adolf, G., Dohlsten, M., Moller, G., & Sjogren, H. O. (1989). Characterization of individual tumor necrosis factor alpha-and beta-producing cells after polyclonal T cell activation. J. Immunol. Meth. 123, 233-240.

Arata, S., Newton, C., Klein, T. W., Yamamoto, Y., & Friedman, H. (1993). Legionella pneumophila induced tumor necrosis factor production in permissive versus nonpermissive macrophages. Proc. Soc. Exp. Biol. Med. 203, 26-29.

Asher, A. L., Mule, J. J., Kasid, A., Restifo, N. P., Salo, J. C., Reichert, C. M., Jaffe, G., Fendly, B., Kriegler, M., & Rosenberg, S. A. (1991). Murine tumor cells transduced with the gene for tumor necrosis factor-alpha. Evidence for paracrine immune effects of tumor necrosis factor against tumors. J. Immunol. 146, 3227-3234.

Atkinson, Y. H., Marasco, W. A., Lopez, A. F., & Vadas, M. A. (1988). Recombinant human tumor necrosis factor-alpha. Regulation of N-formylmethionylleucylphenylalanine receptor affinity and function on human neutrophils. J. Clin. Invest. 81, 759-765.

Austgulen, R., Espevik, T., & Nissen-Meyer, J. (1987). Fibroblast growth-stimulatory activity released from human monocytes. The contribution of tumour necrosis factor. Scand. J. Immunol. 26, 621-629.

Bachwich, P. R., Chensue, S. W., Larrick, J. W., Kunkel, S. L. (1986). Tumor necrosis factor stimulates interleukin-1 and prostaglandin E_2 production in resting macrophages. Biochem. Biophys. Res. Commun. 136, 94-101.

Baker, E., Chen, L. Z., Smith, C. A., Callen, D. F., Goodwin, R., & Sutherland, G. R. (1991). Chromosomal location of the human tumor necrosis factor receptor genes. Cytogenet. Cell Genet. 57, 117-118.

Baker, D., Butler, D., Scallon, B. J., Oneill, J. K., Turk, J. L., & Feldmann, M. (1994). Control of established experimental allergic encephalomyelitis by inhibition of tumor necrosis factor (TNF) activity within the central nervous system using monoclonal antibodies and TNF receptor-immunoglobulin fusion proteins. Eur. J. Immunol. 24, 2040-2048.

Baldwin, R. L., Chang, M. P., Bramhall, J., Graves, S., Bonavida, B., & Wisnieski, B. J. (1988). Capacity of tumor necrosis factor to bind and penetrate membranes is pH-dependent. Scand. J. Immunol. 141, 2352-2357.

Balkwill, F. R., Lee, A., Aldam, G., Moodie, E., Thomas, J. A., Tavernier, J., & Fiers, W. (1986). Human tumor xenografts treated with recombinant human tumor necrosis factor alone or in combination with interferons. Cancer Res. 46, 3990-3993.

Banner, D. W., D'Arcy, A., Janes, W., Gentz, R., Schoenfeld, H. J., Broger, C., Loetscher, H., & Lesslauer, W. (1993). Crystal structure of the soluble human 55 kDa TNF receptor-human TNF beta complex: Implications for TNF receptor activation. Cell 73, 431-445.

Barbara, J. A., Smith, W. B., Gamble, J. R., Van Ostade, X., Vandenabeele, P., Tavernier, J., Fiers, W., Vadas, M. A., & Lopez, A. F. (1994). Dissociation of TNF-alpha cytotoxic and proinflammatory activities by p55 receptor- and p75 receptor-selective TNF-alpha mutants. EMBO J. 13, 843-850.

Bazan, J. F. (1993). Emerging families of cytokines and receptors. Curr. Biol. 3, 603-606.

Benvenuto, R., Paroli, M., Buttinelli, C., Franco, A., Barnaba, V., Fieschi, C., & Balsano, F. (1991). Tumour necrosis factor-alpha synthesis by cerebrospinal-fluid-derived T cell clones from patients with multiple sclerosis. Clin. Exp. Immunol. 84, 97-102.

Beresini, M. H., Lempert, M. J., & Epstein, L. B. (1988). Overlapping polypeptide induction in human fibroblasts in response to treatment with interferon-alpha, interferon-gamma, interleukin 1 alpha, interleukin 1 beta, and tumor necrosis factor. J. Immunol. 140, 485-493.

Bermudez, L. E., & Young, L. S. (1988). Tumor necrosis factor, alone or in combination with IL-2, but not IFN-gamma, is associated with macrophage killing of *Mycobacterium avium* complex. J. Immunol. 140, 3006-3013.

Bermudez, L. E., Wu, M., & Young, L. S. (1990). Effect of stress-related hormones on macrophage receptors and response to tumor necrosis factor. Lymphokine Res. 9, 137-145.

Beutler, B., & Cerami, A. (1989). The biology of cachectin/TNF- a primary mediator of the host response. Annu. Rev. Immunol. 7, 625-655.

Beutler, B., & van Huffel, C. (1994). Unraveling function in the TNF ligand and receptor families. Science 264, 667-668.

Beutler, B., Mahoney, J., Le Trang, N., Pekala, P., & Cerami, A. (1985a). Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. J. Exp. Med. 161, 984-995.

Beutler, B., Milsark, I.W., & Cerami, A. (1985b). Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 229, 869-871.

Beutler, B., Krochin, N., Milsark, I. W., Luedke, C., & Cerami, A. (1989). Control of cachectin (tumor necrosis factor) synthesis: Mechanisms of endotoxin resistance. Science 232, 977-980.

Bevilacqua, M. P., Pober, J. S., Mendick, D. L., Cotran, R. S., & Gimbrone, M. A., Jr. (1987). Identification of an inducible endothelial-leukocyte adhesion molecule. Proc. Natl. Acad. Sci. USA 84, 9238-9248.

Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A., Jr., & Seed, B. (1989). Endothelial leukocyte adhesion molecule 1: An inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science. 243, 1160-1165.

Beyaert, R., & Fiers, W. (1994). Molecular mechanisms of tumor necrosis factor-induced cytotoxicity—What we do understand and what we do not. FEBS Lett. 340, 9-16.

Billard, C., Sigaux, F., & Wietzerbin, J. (1990). IFN-alpha *in vivo* enhances tumor necrosis factor receptor levels on hairy cells. J. Immunol. 145, 1713-1718.

Bigda, J., Beletsky, I., Brakebusch, C., Varfolomeev, Y., Engelmann, H., Bigda, J., Holtmann, H., & Wallach, D. (1994). Dual role of the p75 tumor necrosis factor (TNF) receptor in TNF cytotoxicity. J. Exp. Med. 180, 445-460.

Blanchard, D. K., Djeu, J. Y., Klein, T. W., Friedman, H., & Stewart, W. E., II. (1988). Protective effects of tumor necrosis factor in experimental *Legionella pneumophila* infections of mice via activation of PMN function. J. Leuk. Biol. 43, 429-435.

Blanchard, D. K., Friedman, H., Klein, T. W., & Djeu, J. Y. (1989). Induction of interferon-gamma and tumor necrosis factor by Legionella pneumophila: Augmentation of human neutrophil bactericidal activity. J. Leuk. Biol. 45, 538-545.

Blankenstein, T., Qin, Z. H., Uberla, K., Muller, W., Rosen, H., Volk, H. D., & Diamantstein, T. (1991). Tumor suppression after tumor cell-targeted tumor necrosis factor alpha gene transfer. J. Exp. Med. 173, 1047-1052.

Blenis, J. (1993). Signal transduction via the MAP kinases: Proceed at your own RSK. Proc. Natl. Acad. Sci. USA 90, 5889-5892.

Boldin, M.P., Goncharov, T. M., Goltsev, Y. V., & Wallach, D. (1996). Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85, 803-815.

Boussiotis, V. A., Nadler, L. M., Strominger, J. L., & Goldfield, A. E. (1994). Tumor necrosis factor {\SYMBOL 97 \f "Symbol"} is an autocrine growth factor for normal human B cells. Proc. Natl. Acad. Sci. USA 91, 7007-7011.

Bradbury, M. G., & Moreno, C. (1993). Effect of lipoarabinomannan and mycobacteria on tumour necrosis factor production by different populations of murine macrophages. Clin. Exp. Immunol. 94, 57-63.

Brahn, E., Peacock, D. J., Banquerigo, M. L., & Liu, D. Y. (1992). Effects of tumor necrosis factor alpha (TNF-alpha) on collagen arthritis. Lymphokine Cytokine Res. 11, 253-256.

Brakebusch, C., Nophar, Y., Kemper, O., Engelmann, H., & Wallach, D. (1992). Cytoplasmic truncation of the p55 tumour necrosis factor (TNF) receptor abolishes signalling, but not induced shedding of the receptor. EMBO J. 11, 943-950.

Breder, C. D., Hazuka, C., Ghayur, T., Klug, C., Huiginin, M., Yasuda, K., Teng, M., & Saper, C. B. (1994). Regional induction of tumor necrosis Factor Alpha expression in the mouse brain after systemic lipopolysaccharide administration. Proc. Natl. Acad. Sci. USA 91, 11393-11397.

Brennan, F. M., Chantry, D., Jackson, A., Maini, R., & Feldmann, M. (1989). Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet. 2, 244-247.

Brockhaus, M., Schoenfeld, H. J., Schlaeger, E. J., Hunziker, W., Lesslauer, W., & Loetscher, H. (1990). Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc. Natl. Acad. Sci. USA 87, 3127-3131.

Butcher, G. A., Garland, T., Ajdukiewicz, A. B., & Clark, I. A. (1990). Serum tumor necrosis factor associated with malaria in patients in the Solomon Islands. Trans. R. Soc. Trop. Med. Hyg. 84, 658-661.

Cadranel, J., Philippe, C., Perez, J., Milleron, B., Akoun, G., Ardaillou, R., & Baud, L. (1990). In vitro production of tumour necrosis factor and prostaglandin E2 by peripheral blood mononuclear cells from tuberculosis patients. Clin. Exp. Immunol. 81, 319-324.

Camussi, G., Bussolino, F., Salvidio, G., & Baglioni, C. (1987). Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. J. Exp. Med. 166, 1390-1404.

Camussi, G., Tetta, C., Bussolino, F., Turello, E., Brentjens, J., Montrucchio, G., & Andres, G. (1990). Effect of leukocyte stimulation on rabbit immune complex glomerulonephritis. Kidney Int. 38, 1047-1055.

Cannistra, S. A., Vellenga, E., Groshek, P., Rambaldi, A., & Griffin, J. D. (1988). Human granulocyte-monocyte colony-stimulating factor and interleukin 3 stimulate monocyte cytotoxicity through a tumor necrosis factor-dependent mechanism. Blood. 71, 672-676.

Carroll, M. C., Katzman, P., Alicot, E. M., Koller, B. H., Geraghty, D. E., Orr, H. T., Strominger, J. L., & Spies, T. (1987). Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. Proc. Natl. Acad. Sci. USA 84, 8535-8539.

Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., & Williamson, B. (1975). An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA 72, 3666-3670.

Chambaut-Guerin, A. M., & Thomopoulos, P. (1991). Regulation by dexamethasone and 1,25-dihydroxyvitamin D3 of the 75 kD-type A tumor necrosis factor receptor surface expression and mRNA level in human U937 cells. Eur. Cytokine Netw. 2, 355-360.

Chantry, D., Turner, M., Abney, E., & Feldmann, M. (1989). Modulation of cytokine production by transforming growth factor-beta. J. Immunol. 142, 4295-4300.

Chen, S. H., Oakes, J. E., & Lausch, R. N. (1993). Synergistic anti-HSV effect of tumor necrosis factor alpha and interferon gamma in human corneal fibroblasts is associated with interferon beta induction. Antiviral. Res. 22, 15-29.

Cheng, G., & Baltimore, D. (1996). TANK, a co-inducer with TRAF-2 of TNF- and CD40L-mediated NF-κB activation. Genes Develop. 10, 963-973.

Chollet-Martin, S., Montravers, P., Gibert, C., Elbim, C., Desmonts, J. M., Fagon, J. Y., & Gougerot-Pocidalo, M. A. (1993). High levels of interleukin-8 in the blood and alveolar spaces of patients with pneumonia and adult respiratory distress syndrome. Infect. Immun. 61, 4553-4559.

Chu, C. Q., Field, M., Feldmann, M., & Maini, R. N. (1991). Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum. 34, 1125-1132.

Clark, I. A., Cowden, W. B., Butcher, G. A., & Hunt, N. H. (1987). Possible roles of tumor necrosis factor in the pathology of malaria. American. J. Pathol. 129, 192-199.

Clauss, M., Ryan, J., & Stern, D. (1992). Modulation of endothelial cell hemostatic properties by TNF: Insights into the role of endothelium in the host response to inflammatory stimuli. In: *Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine* (Beutler, B., Ed.). Raven Press, New York, pp. 49-63.

Coley, W. B. (1893). The treatment of malignent tumors by repeated inoculations of erysipelas; with a report of ten original cases. Am. J. Med. Sci. 105, 487-511.

Coley, W. B. (1896). The therapeutic value of the mixed toxins of the streptococcus of erysipelas ih the treatment of inoperable malignant tumors, with a report of 100 cases. Am. J. Med. Sci. 112, 251-281.

Cornelius, P., Enerback, S., Bjursell, G., Olivecrona, T., & Pekala, P. H. (1988). Regulation of lipoprotein lipase mRNA content in 3T3-L1 cells by tumour necrosis factor. Biochem. J. 249, 765-769.

Corti, A., Fassina, G., Marcucci, F., Barbanti, E., & Cassani, G. (1992). Oligomeric tumour necrosis factor alpha slowly converts into inactive forms at bioactive levels. Biochem. J. 284, 905-910.

Creasey, A. A., Reynolds, M. T., & Laird, W. (1986). Cures and partial regression of murine and human tumors by recombinant human tumor necrosis factors. Cancer Res. 46, 5687-5690.

Crowe, P. D., Van Arsdale, T. L., Goodwin, R. G., & Ware, C. F. (1993). Specific induction of 80-kDa tumor necrosis factor receptor shedding in T-Lymphocytes involves the cytoplasmic domain and phosphorylation. J. Immunol. 151, 6882-6890.

Crowe, P. D., Van Arsdale, T. L., Walter, B. N., Ware, C. F., Hession, C., Ehrenfels, B., Browning, J. L., Din, W. S., Goodwin, R. G., & Smith, C. A. (1994). A lymphotoxin-beta-specific receptor. Science 264, 707-710.

Cseh, K., & Beutler, B. (1989). Alternative cleavage of the cachectin/tumor necrosis factor propeptide results in a larger, inactive form of secreted protein. J. Biol. Chem. 264, 16256-16260.

Cuturi, M. C., Murphy, M., Costa-Giomi, M. P., Weinmann, R., Perussia, B., & Trinchieri, G. (1987). Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. J. Exp. Med. 165, 1581-1594.

Darnay, B. G., Reddy, S. A. G., & Aggarwal, B. B. (1994a). Identification of a protein kinase associated with the cytoplasmic domain of the p60 tumor necrosis factor receptor. J. Biol. Chem. 269, 20299-20304.

Darnay, B. G., Reddy, S. A. G., & Aggarwal, B. B. (1994b). Physical and functional association of a serine-threonine protein kinase to the cytoplasmic domain of the p80 form of the human tumor necrosis factor receptor in human histiocytic lymphoma U-937 cells. J. Biol. Chem. 269, 19687-19690.

Dayer, J. M. (1991). Chronic inflammatory joint diseases: Natural inhibitors of interleukin 1 and tumor necrosis factor alpha. J. Rheumatol. Suppl. 27, 71-75.

De Valck, D., Beyaert, R., Van Roy, F., & Fiers, W. (1993). Tumor necrosis factor cytotoxicity is associated with phospholipase D activation. Eur. J. Biochem. 212, 491-497.

Desiderio, J. V., Kiener, P. A., Lin, P. F., & Warr, G. A. (1989). Protection of mice against Listeria monocytogenes infection by recombinant human tumor necrosis factor alpha. Infect. Immun. 57, 1615-1617.

Dett, C. A., Gatanaga, M., Ininnis, E. K., Cappuccini, F., Yamamoto, R. S., Granger, G. A., & Gatanaga, T. (1991). Enhancement of lymphokine-activated T killer cell tumor necrosis factor receptor mRNA transcription, tumor necrosis factor receptor membrane expression, and tumor necrosis factor/lymphotoxin release by IL-1 beta, IL-4, and IL-6 *in vitro*. J. Immunol. 146, 1522-1526.

Diazmeco, M. T., Berra, E., Municio, M. M., Sanz, L., Lozano, J., Dominguez, I., Diazgolpe, V., Delera, M. T. L., Alcami, J., Paya, C. V., Arenzanaseisdedos, F., Virelizier, J. L., & Moscat, J. (1993). A dominant negative protein kinase-C zeta-subspecies blocks NF-kappa-B activation. Mol. Cell. Biol. 13, 4770-4775.

Digel, W., Schoniger, W., Stefanic, M., Janssen, H., Buck, C., Schmid, M., Raghavachar, A., & Porzsolt, F. (1990). Receptors for tumor necrosis factor on neoplastic B cells from chronic lymphocytic leukemia are expressed *in vitro* but not *in vivo*. Blood 76, 1607-1613.

Dinarello, C. A., Cannon, J. G., Wolff, S. M., Bernheim, H. A., Beutler, B., Cerami, A., Figari, F. S., Palladino, M. A., & O'Connor, J. V. (1986). Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J. Exp. Med. 163, 1433-1450.

Dinarello, C. A., Gelfand, J. A., & Wolff, S. M. (1993). Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. Jama 269, 1829-1835.

Djeu, J. Y. (1992). TNF production by neutrophils. In: *Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine* (Beutler, B., Ed.). Raven Press, New York, pp. 531-537.

Dobrowsky, R. T., & Hannun, Y. A. (1992). Ceramide stimulates a cytosolic protein phosphatase. J. Biol. Chem. 267, 5048-5051.

Duerksen-Hughes, P., Wold, W. S., & Gooding, L. R. (1989). Adenovirus E1A renders infected cells sensitive to cytolysis by tumor necrosis factor. J. Immunol. 143, 4193-4200.

Eck, M. J., & Sprang, S. R. (1989). The structure of tumor necrosis factor-alpha at 2.6 Å resolution. Implications for receptor binding. J. Biol. Chem. 264, 17595-17605.

Eck, M. J., Beutler, B., Kuo, G., Merryweather, J. P., & Sprang, S. R. (1988). Crystallization of trimeric recombinant human tumor necrosis factor (cachectin). J. Biol. Chem. 263, 12816-12819.

Eck, M. J., Ultsch, M., Rinderknecht, E., de Vos, A. M., & Sprang, S. R. (1992). The structure of human lymphotoxin (tumor necrosis factor-beta) at 1.9-Å resolution. J. Biol. Chem. 267, 2119-2122.

Economou, J. S., Rhoades, K., Essner, R., McBride, W. H., Gasson, J. C., & Morton, D. L. (1989). Genetic analysis of the human tumor necrosis factor alpha/cachectin promoter region in a macrophage cell line. J. Exp. Med. 170, 321-326.

Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Katsikis, P., Brennan, F. M., Walker, J., Bijl, H., Ghrayeb, J., & Woody, J.W. (1993). Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. Arthritis Rheum. 36, 1681-1690.

Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., Smolen, J. S., Leeb, B., Breedveld, F. C., Macfarlane, J. D., Bijl, H., & Woody, J. N. (1994a). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 344, 1105-1110.

Elliott, M. J., Maini, R. N., Feldmann, M., Longfox, A., Charles, P., Bijl, H., & Woody, J. N. (1994b). Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. Lancet 344, 1125-1127.

Engelmann, H., Holtmann, H., Brakebusch, C., Avni, Y. S., Sarov, I., Nophar, Y., Hadas, E., Leitner, O., & Wallach, D. (1990a). Antibodies to a soluble form of a tumor necrosis factor (TNF) receptor have TNF-like activity. J. Biol. Chem. 265, 14497-14504.

Engelmann, H., Novick, D., & Wallach, D. (1990b). Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J. Biol. Chem. 265, 1531-1536.

Erickson, S., de Sauvage, F. J., Kikly, K., Carver-Moore, K., Pitts-Meek, S., Gillett, N., Sheehan, K. C. F., Schreiber, R. D., Goeddel, D. V., & Moore, M. W. (1994). Decreased sensitivity to tumour-necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. Nature 372, 560-563.

Erikstein, B. K., Smeland, E. B., Blomhoff, H. K., Funderud, S., Prydz, K., Lesslauer, W., & Espevik, T. (1991). Independent regulation of 55-kDa and 75-kDa tumor necrosis factor receptors during activation of human peripheral blood B lymphocytes. Eur. J. Immunol. 21, 1033-1037.

Espevik, T., Figari, I. S., Ranges, G. E., & Palladino, M. A., Jr. (1988). Transforming growth factor-beta 1 (TGF-beta 1) and recombinant human tumor necrosis factor-alpha reciprocally regulate the generation of lymphokine-activated killer cell activity. Comparison between natural porcine platelet-derived TGF-beta 1 and TGF-beta 2, and recombinant human TGF-beta 1. J. Immunol. 140, 2312-2316.

Evans, T. J., Moyes, D., Carpenter, A., Martin, R., Loetscher, H., Lesslauer, W. & Cohen, J. (1994). Protective effect of 55- but not 75-kD soluble tumor necrosis factor receptor-immunoglobulin G fusion proteins in an animal model of gram-negative sepsis. J. Exp. Med. 180, 2173-2179.

Fahey, T. J., III., Sherry, B., Tracey, K. J., van Deventer, S., Jones, W. G., II., Minei, J. P., Morgello, S., Shires, G. T., & Cerami, A. (1990). Cytokine production in a model of wound healing: The appearance of MIP-1, MIP-2, cachectin/TNF and IL-1. Cytokine 2, 92-99.

Feldmann, M., Brennan, F. M., Williams, R. O., Cope, A. P., Gibbons, D. L., Katsikis, P. D., & Maini, R. N. (1992). Evaluation of the role of cytokines in autoimmune disease: the importance of TNF alpha in rheumatoid arthritis. Prog. Growth Factor Res. 4, 247-255.

Ferran, C., Dautry, F., Merite, S., Sheeman, K., Schreiber, R., Grau, G., Bach, J.-F., & Chatenoud, L. (1994). Anti-tumor necrosis factor modulates anti-CD3-triggerd T cell cytokine gene expression *in vivo.* J. Clin. Invest. 93, 2189-2196.

Fishman, M. (1991). Cytolytic activities of activated macrophages versus paraformaldehyde-fixed macrophages; soluble versus membrane-associated TNF. Cell. Immunol. 137, 164-174.

Franciotta, D. M., Grimaldi, L. M., Martino, G. V., Piccolo, G., Bergamaschi, R., Citterio, A., & Melzi d'Eril, G. V. (1989). Tumor necrosis factor in serum and cerebrospinal fluid of patients with multiple sclerosis. Ann. Neurol. 26, 787-789.

Fraser, A., & Evan, G. (1996). A license to kill. Cell 85, 781-784.

Frei, E., III, & Spriggs, D. (1989). Tumor necrosis factor: Still a promising agent. J. Clin. Oncol. 7, 291-294.

Freund, Y. R., Sgarlato, G., Jacob, C. O., Suzuki, Y., & Remington, J. S. (1992). Polymorphisms in the tumor necrosis factor alpha (TNF-alpha) gene correlate with murine resistance to development of toxoplasmic encephalitis and with levels of TNF-alpha mRNA in infected brain tissue. J. Exp. Med. 175, 683-688.

Fuchs, P., Strehl, S., Dworzak, M., Himmler, A., & Ambros, P. F. (1992). Structure of the human TNF receptor 1 (p60) gene (TNFR1) and localization to chromosome 12p13. Genomics 13, 219-224.

Furman, W. L., Strother, D., McClain, K., Bell, B., Leventhal, B., & Pratt, C. B. (1993). Phase I clinical trial of recombinant human tumor necrosis factor in children with refractory solid tumors: A Pediatric Oncology Group study. J. Clin. Oncol. 11, 2205-2210.

Gamble, J. R., Harlan, J. M., Klebanoff, S. J., & Vadas, M. A. (1985). Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc. Natl. Acad. Sci USA 82, 8667-8671.

Gamble, J. R., Smith, W. B., & Vadas, M. A. (1992). TNF modulation of endothelial and neutrophil adhesion. In: *Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine* (Beutler, B., Ed.). Raven Press, New York, pp. 65-86.

Gatanaga, T., Hwang, C. D., Kohr, W., Cappuccini, F., Lucci, J. A., III., Jeffes, E. W., Lentz, R., Tomich, J., Yamamoto, R. S., & Granger, G. A. (1990). Purification and characterization of an inhibitor (soluble tumor necrosis factor receptor) for tumor necrosis factor and lymphotoxin obtained from the serum ultrafiltrates of human cancer patients. Proc. Natl. Acad. Sci. USA 87, 8781-8784.

Gatanaga, T., Hwang, C. D., Gatanaga, M., Cappuccini, F., Yamamoto, R. S., & Granger, G. A. (1991). The regulation of TNF receptor mRNA synthesis, membrane expression, and release by PMA- and LPS-stimulated human monocytic THP-1 cells *in vitro*. Cell. Immunol. 138, 1-10.

Gearing, A. J. H., Beckett, P., Christodoulou, M., Churchill, M., Clements, J., Davidson, A. H., Drummond, A. H., Galloway, W. A., Gilbert, R., Gordon, J. L., Leber, T. M., Mangan, M., Miller, K., Nayee, P., Owen, K., Patel, S., Thomas, W., Wells, G., Wood, L. M., & Woolley, K. (1994). Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature 370, 555-557.

Gehr, G., Gentz, R., Brockhaus, M., Loetscher, H., & Lesslauer, W. (1992). Both tumor necrosis factor receptor types mediate proliferative signals in human mononuclear cell activation. J. Immunol. 149, 911-917.

Gessani, S., Johnson, S. E., McCandless, S., & Baglioni, C. (1988). The antiviral activity of tumor necrosis factor in HeLa cells is not mediated by interferons. Studies with TNF-resistant variants. J. Biol. Regul. Homeost. Agents 2, 139-144.

Ghiara, P., Boraschi, D., Nencioni, L., Ghezzi, P., & Tagliabue, A. (1987). Enhancement of in vivo immune response by tumor necrosis factor. J. Immunol. 139, 3676-3679.

Gifford, G. E., & Lohmann-Matthes, M. L. (1987). Gamma interferon priming of mouse and human macrophages for induction of tumor necrosis factor production by bacterial lipopolysaccharide. J. Natl. Cancer Inst. 78, 121-124.

Goodwin, R. G., Anderson, D., Jerzy, R., Davis, T., Brannan, C. I., Copeland, N. G., Jenkins, N. A., & Smith, C. A. (1991). Molecular cloning and expression of the type 1 and type 2 murine receptors for tumor necrosis factor. Mol. Cell. Biol. 11, 3020-3026.

Grau, G. E., Fajardo, L. F., Piquet, P.-F., Allet, B., Lambert, P.-H., & Vasalli, P. (1987). Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science 237, 1210-1212.

Grau, G. E., Heremans, H., Piguet, P. F., Pointaire, P., Lambert, P. H., Billiau, A., & Vassalli, P. (1989a). Monoclonal antibody against interferon gamma can prevent experimental cerebral malaria and its associated overproduction of tumor necrosis factor. Proc. Natl. Acad. Sci. USA 86, 5572-5574.

Grau, G. E., Taylor, T. E., Molyneux, M. E., Wirima, J. J., Vassalli, P., Hommel, M., & Lambert, P. H. (1989b). Tumor necrosis factor and disease severity in children with falciparum malaria. N. Engl. J. Med. 320, 1586-1591.

Grell, M., Scheurich, P., Meager, A., & Pfizenmaier, K. (1993). TR60 and TR80 tumor necrosis factor (TNF)-receptors can independently mediate cytolysis. Lymphokine Cytokine Res. 12, 143-148.

Grell, M., Zimmermann, G., Hülser, D., Pfizenmaier, K., & Scheurich, P. (1994). TNF receptors TR60 and TR80 can mediate apoptosis via induction of distinct signal pathways. J. Immunol. 153, 1963-1972.

Grell, M., Douni, E., Wajant, H., Löhden, M., Clauss, M., Maxeiner, B., Georgopoulos, S., Lesslauer, W., Kollias, G., Pfizenmaier, K., & Scheurich, P. (1995). The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDA tumor necrosis factor receptor. Cell 83, 793-802.

Gresser, I., Belardelli, F., Maury, C., Tovey, M. G., & Maunoury, M. T. (1986). Anti-tumor effects of interferon in mice injected with interferon-sensitive and interferon-resistant friend leukemia cells. Int. J. Cancer 38, 251-257.

Guy, G. R., Cao, X., Chua, S. P., & Tan, Y. H. (1992). Okadaic acid mimics multiple changes in early protein phosphorylation and gene expression induced by tumor necrosis factor or interleukin-1. J. Biol. Chem. 267, 1846-1852.

Hackett, R. J., Davis, L. S., & Lipsky, P. E. (1988). Comparative effects of tumor necrosis factor-alpha and IL-1 beta on mitogen-induced T cell activation. J. Immunol. 140, 2639-2644.

Haliday, E. M., Ramesha, C. S., & Ringold, G. (1991). TNF induces c-fos via a novel pathway requiring conversion of arachidonic acid to a lipoxygenase metabolite. EMBO J. 10, 109-115.

Han, J., Thompson, P., & Beutler, B. (1990). Dexamethasone and pentoxifylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. J. Exp. Med. 172, 391-394.

Hanemaaijer, R., Koolwijk, P., le Clercq, L., de Vree, W. J., & van Hinsbergh, V. W. (1993). Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. Biochem. J. 296, 803-809.

Hannun, Y. A. (1994). The sphingomyelin cycle and the second messenger function of ceramide. J. Biol. Chem. 269, 3125-3128.

Haranaka, K. (1988). Tumor necrosis factor: How to improve the antitumor activity and decrease accompanying side effects for therapeutic application. J. Biol. Response Mod. 7, 525-534.

Haskard, D., Cavender, D., Beatty, P., Sprninger, T., & Ziff, M. (1986). T lymphocyte adhesion to endothelial cells: Mechanisms demonstrated by anti-LFA-1 antibodies. J. Immunol 137, 2901-2906.

Hauser, S. L., Doolittle, T. H., Lincoln, R., Brown, R. H., & Dinarello, C. A. (1991). Cytokine accumulations in CSF of multiple sclerosis patients: Frequent detection of IL-1 and TNF but not IL-6. Neurology 40, 1735-1739.

Havell, E. A. (1989). Evidence that tumor necrosis factor has an important role in antibacterial resistance. J. Immunol. 143, 2894-2899.

Havell, E. A. (1993). Listeria monocytogenes-induced interferon-gamma primes the host for production of tumor necrosis factor and interferon-alpha/beta. J. Infect. Dis. 167, 1364-1371.

Havell, E. A., Fiers, W., & North, R. J. (1988). The antitumor function of tumor necrosis factor (TNF), I. Therapeutic action of TNF against an established murine sarcoma is indirect, immunologically dependent, and limited by severe toxicity. J. Exp. Med. 167, 1967-1985.

Hayakawa, M., Ishida, N., Takeuchi, K., Shibamoto, S., Hori, T., Oku, N., Ito, F., & Tsujimoto, M. (1993). Arachidonic acid-selective cytosolic phospholipase A_2 is crucial in the cytotoxic action of tumor necrosis factor. J. Biol. Chem. 268, 11290-11295.

Heller, R. A., & Krönke, M. (1994). Tumor necrosis factor receptor-mediated signaling pathways. J. Cell Biol. 126, 5-9.

Heller, R. A., Song, K., Fan, N., & Chang, D. J. (1992). The p70 tumor necrosis factor receptor mediates cytotoxicity. Cell 70, 47-56.

Hensel, G., Meichle, A., Pfizenmaier, K., & Krönke, M. (1989). PMA-responsive 5' flanking sequences of the human TNF gene. Lymphokine Res. 8, 347-351.

Higuchi, M., & Aggarwal, B. B. (1993). Okadaic acid induces down-modulation and shedding of tumor necrosis factor receptors. Comparison with another tumor promoter, phorbol ester. J. Biol. Chem. 268, 5624-5631.

Higuchi, M., & Aggarwal, B. B. (1994). TNF induces internalization of the p60 receptor and shedding of the p80 receptor. J. Immunol. 152, 3550-3558.

Himmler, A., Maurer-Fogy, I., Krönke, M., Scheurich, P., Pfizenmaier, K., Lantz, M., Olsson, I., Hauptmann, R., Stratowa, C., & Adolf, G. R. (1990). Molecular cloning and expression of human and rat tumor necrosis factor receptor chain (p60) and its soluble derivative, tumor necrosis factor-binding protein. DNA Cell. Biol. 9, 705-715.

Hohmann, H. P., Remy, R., Brockhaus, M., & van Loon, A. P. G. M. (1989). Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha). J. Biol. Chem. 264, 14927-14934.

Hori, T., Kashiyama, S., Oku, N., Hayakawa, M., Shibamoto, S., Tsujimoto, M., Nishihara, T., & Ito, F. (1988). Effects of tumor necrosis factor on cell growth and expression of transferrin receptors in human fibroblasts. Cell Struct. Funct. 13, 425-433.

Howard, O. M. Z., Clouse, K. A., Smith, C., Goodwin, R. G., & Farrar, W. L. (1993). Soluble tumor necrosis factor receptor—inhibition of human immunodeficiency virus activation. Proc. Natl. Acad. Sci. USA 90, 2335-2339.

Hsu, H., Xiong, J., & Goeddel, D. V. (1995). The TNF receptor 1-associated protein TRADD signals cell death and NF-κB activation. Cell 81, 495-504.

Hsu, H., Shu, H.-B., Pan, M.-G., & Goeddel, D. V. (1996a). TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell 84, 299-308.

Hsu, H., Huang, J., Shu, H.-B., Baichwal, V., & Goeddel, D. V. (1996b). TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4, 387-396.

Hu, F.-Q., Goodwin, R. G., Davis, T., Pickup, D. J., & Smith, C. A. (1994). Cowpox virus encodes two soluble, secreted forms of TNF receptor. Eur. Cytokine Netw. 5, 125.

Hughes, T. K., Kaspar, T. A., & Coppenhaver, D. H. (1988). Synergy of antiviral actions of TNF and IFN-gamma: Evidence for a major role of TNF-induced IFN-beta. Antiviral. Res. 10, 1-9.

Israel, N., Hazan, U., Alcami, J., Munier, A., Arenzana-Seisdedos, F., Bachelerie, F., Israel, A., & Virelizier, J. L. (1989). Tumor necrosis factor stimulates transcription of HIV-1 in human T lymphocytes, independently and synergistically with mitogens. J. Immunol. 143, 3956-3960.

Itoh, N., Yonehara, S., Ishi, A., Yonehara, M., Mizushima, S., Sameshima, M., Hase, A., Seto, Y., & Nagata, S. (1991). The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 66, 233-243.

Jäättelä, M. (1991). Biologic activities and mechanisms of action of tumor necrosis factor-alpha/cachectin. Lab. Invest. 64, 724-742.

Jäättelä, M. (1993). Overexpression of major heat shock protein hsp70 inhibits tumor necrosis factor-induced activation of phospholipase A_2. J. Immunol. 151, 4286-4294.

Jäättelä, M., & Wissing, D. (1993). Heat-shock proteins protect cells from monocyte cytotoxicity: possible mechanism of self-protection. J. Exp. Med. 177, 231-236.

Jacob, C. O., & McDevitt, H. O. (1988). Tumour necrosis factor-alpha in murine autoimmune "lupus" nephritis. Nature. 331, 356-358.

Jelinek, D. F., & Lipsky, P. E. (1987). Enhancement of human B cell proliferation and differentiation by tumor necrosis factor-alpha and interleukin 1. J. Immunol. 139, 2970-2976.

Jevnikar, A. M., Brennan, D. C., Singer, G. G., Heng, J. E., Maslinski, W., Wuthrich, R. P., Glimcher, L. H., & Kelley, V. E. (1991). Stimulated kidney tubular epithelial cells express membrane associated and secreted TNF alpha. Kidney Int. 40, 203-211.

Johnson, D. R., & Pober, J. S. (1990). Tumor necrosis factor and immune interferon synergistically increase transcription of HLA class I heavy- and light-chain genes in vascular endothelium. Proc. Natl. Acad. Sci. USA 87, 5183-5187.

Jones, E. Y., Stuart, D. I., & Walker, N. P. (1989). Structure of tumour necrosis factor. Nature. 338, 225-228.

Jongeneel, C. V. (1992). The TNF and lymphotoxin promoters. In: *Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine* (Beutler, B., Ed.). Raven Press, New York, pp. 539-559.

Kagan, B. L., Baldwin, R. L., Munoz, D., & Wisnieski, B. J. (1992). Formation of ion-permeable channels by tumor necrosis factor-alpha. Science 255, 1427-1430.

Kalthoff, H., Roeder, C., Brockhaus, M., Thiele, H. G., & Schmiegel, W. (1993). Tumor necrosis factor (TNF) up-regulates the expression of p75 but not p55 TNF receptors, and both receptors mediate, independently of each other, up-regulation of transforming growth factor alpha and epidermal growth factor receptor mRNA. J. Biol. Chem. 268, 2762-2766.

Kasid, A., Director, E. P., Stovroff, M. C., Lotze, M. T., & Rosenberg, S. A. (1990). Cytokine regulation of tumor necrosis factor-alpha and -beta (lymphotoxin)-messenger RNA expression in human peripheral blood mononuclear cells. Cancer Res. 50, 5072-5076.

Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., & Kollias, G. (1991). Transgenic mice expressing human tumour necrosis factor: A predictive genetic model of arthritis. EMBO J. 10, 4025-4031.

Kemeny, N., Childs, B., Larchian, W., Rosado, K., & Kelsen, D. (1990). A phase II trial of recombinant tumor necrosis factor in patients with advanced colorectal carcinoma. Cancer 66, 659-663.

Kern, P., Hemmer, C. J., Van Damme, J., Gruss, H. J., & Dietrich, M. (1989). Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated Plasmodium falciparum malaria. Am. J. Med. 87, 139-143.

Kim, M. Y., Linardic, C., Obeid, L., & Hannun, Y. (1991). Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation. J. Biol. Chem. 266, 484-489.

Kindler, V., Sappino, A. P., Grau, G. E., Piguet, P. F., & Vassalli, P. (1989). The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell 56, 731-740.

Klebanoff, S. J., Vadas, S. A., Harlan, J. M., Sparks, L. H., Gamble, J. R., Agosi, J. M., & Waltersdorph, A. M. (1986). Stimulation of neutrophils by tumor necrosis factor. J. Immunol. 136, 4220-4225.

Knight, D. M., Trinh, H., Le, J., Siegel, S., Shealy, D., McDonough, M., Scallon, B., Moore, M. A., Vilcek, J., Daddona, P., & Ghrayeb, J. (1993). Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. Mol. Immunol. 30, 1443-1453.

Kock, A., Schwarz, T., Kirnbauer, R., Urbanski, A., Perry, P., Ansel, J. C., & Luger, T. A. (1990). Human keratinocytes are a source for tumor necrosis factor alpha: Evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J. Exp. Med. 172, 1609-1614.

Kohno, M., Nishizawa, N., Tsujimoto, M., & Nomoto, H. (1990). Mitogenic signalling pathway of tumour necrosis factor involves the rapid tyrosine phosphorylation of 41,000-M_r and 43,000-M_r cytosol proteins. Biochem. J. 267, 91-98.

Kolesnick, R., & Golde, D. W. (1994). The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 77, 325-328.

Kollias, G. (1993). Tumour necrosis factor: A specific trigger in arthritis. Res. Immunol. 144, 342-347.

Kolls, J., Peppel, K., Silva, M., & Beutler, B. (1994). Prolonged and effective blockade of tumor necrosis factor activity through adenovirus-mediated gene transfer. Proc. Natl. Acad. Sci. USA 91, 215-219.

Kongshavn, P. A., & Ghadirian, E. (1988). Effect of tumor necrosis factor on growth of *Trypanosoma musculi in vivo* and *in vitro*. Adv. Exp. Med. Biol. 239, 257-262.

Kriegler, M., Perez, C., DeFay, K., Albert, I., & Lu, S. D. (1988). A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: Ramifications for the complex physiology of TNF. Cell 53, 45-53.

Krönke, M., Hensel, G., Schlüter, C., Scheurich, P., Schütze, S., & Pfizenmaier, K. (1988). Tumor necrosis factor and lymphotoxin gene expression in human tumor cell lines. Cancer Res. 48, 5417-5421.

Krönke, M., Schütze, S., Scheurich, P., & Pfizenmaier, K. (1992). TNF signal transduction and TNF-responsive genes. In: *Tumor Necrosis Factors: Structure, Function and Mechanism of Action* (Aggarwal, B. B., & Vilcek, J., Eds.). Marcel Dekker Inc., New York, pp. 189-216.

Kunitani, M. G., Cunico, R. L., & Staats, S. J. (1988). Reversible subunit dissociation of tumor necrosis factor during hydrophobic interaction chromatography. J. Chromatogr. 443, 205-220.

Kuprash, D., Turetskaya, R., Boldin, M., Udalova, I., Kistanova, E., Smirnova, J., & Nedospasov, S. (1993). Structure, polymorphism and regulation of tumor necrosis factor genes at genomic loci. In: *Tumor Necrosis Factor: Molecular and Cellular Biology and Clinical Relevance* (Fiers, W., & Buurman, W. A., Eds.). Karger, Basel, Switzerland, pp. 19-26.

Kwiatkowski, D. (1993). TNF-inducing malaria toxin: A sheep in wolf's clothing? Ann. Trop. Med. Parasitol. 87, 613-616.

Kwiatkowski, D., Molyneux, M. E., Stephens, S., Curtis, N., Klein, N., Pointaire, P., Smit, M., Allan, R., Brewster, D. R., Grau, G. E., & Greenwood, B. M. (1993). Anti-TNF therapy inhibits fever in cerebral malaria. Q. J. Med. 86, 91-98.

Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., & Woodgett, J. R. (1994). The stress-activated protein kinase subfamily of c-Jun kinases. Nature 369, 165-160.

Lancaster, J. R., Jr., Laster, S. M., & Gooding, L. R. (1989). Inhibition of target cell mitochondrial electron transfer by tumor necrosis factor. FEBS Lett. 248, 169-174.

Langermans, J. A., van der Hulst, M. E., Nibbering, P. H., & van Furth, R. (1992). Endogenous tumor necrosis factor alpha is required for enhanced antimicrobial

activity against *Toxoplasma gondii* and *Listeria monocytogenes* in recombinant gamma interferon-treated mice. Infect. Immun. 60, 5107-5112.

Lantz, M., Gullberg, U., Nilsson, E., & Olsson, I. (1990). Characterization *in vitro* of a human tumor necrosis factor-binding protein. A soluble form of a tumor necrosis factor receptor. J. Clin. Invest. 86, 1336-1342.

Lantz, M., Thysell, H., Nilsson, E., & Olsson, I. (1991). On the binding of tumor necrosis factor (TNF) to heparin and the release *in vivo* of the TNF-binding protein I by heparin. J. Clin. Invest. 88, 2026-2031.

Laster, S. M., Wood, J. G., & Gooding, L. R. (1988). Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J. Immunol. 141, 2629-2634.

Lee, J. C., Truneh, A., Smith, M. F., & Tsang, K. J. (1987). Indcution of interleukin 2 receptor (TAC) by tumor necrosis factor in YT cells. J. Immunol. 139, 1935-1938.

Lee, M. D., Zentella, A., Pekala, P. H., & Cerami, A. (1987). Effect of endotoxin-induced monokines on glucose metabolism in the muscle cell line L6. Proc. Natl. Acad. Sci. USA 84, 2590-2594.

Lee, T. H., Lee, G. W., Ziff, E. B., & Vilcek, J. (1990). Isolation and characterization of eight tumor necrosis factor-induced gene sequences from human fibroblasts. Mol. Cell. Biol. 10, 1982-1988.

Lee, T. H., Wisniewski, H. G., & Vilcek, J. (1992). A novel secretory tumor necrosis factor-inducible protein (TSG-6) is a member of the family of hyaluronate binding proteins, closely related to the adhesion receptor CD44. J. Cell Biol. 116, 545-557.

Lee, G. W., Lee, T. H., & Vilcek, J. (1993). TSG-14, a tumor necrosis factor- and IL-1-inducible protein, is a novel member of the pentaxin family of acute phase proteins. J. Immunol. 150, 1804-1812.

Lejeune, F., Lienard, D., Eggermont, A., Koops, H. S., Rosenkaimer, F., Gerain, J., Klaase, J., Kroon, B., Vanderveken, J., & Schmitz, P. (1994). Rationale for using TNF alpha and chemotherapy in regional therapy of melanoma. J. Cell. Biochem. 56, 52-61.

Lewis, M., Tartaglia, L. A., Lee, A., Bennett, G. L., Rice, G. C., Wong, G. H., Chen, E. Y., & Goeddel, D. V. (1991). Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. Proc. Natl. Acad. Sci. USA 88, 2830-2834.

Libert, C., Takahashi, N., Cauwels, A., Brouckaert, P., Bluethmann, H., & Fiers, W. (1994). Response of interleukin-6-deficient mice to tumor necrosis factor-induced metabolic changes and lethality. Eur. J. Immunol. 24, 2237-2242.

Lienard, D., Ewalenko, P., Delmotte, J. J., Renard, N., & Lejeune, F. J. (1992a). High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. J. Clin. Oncol. 10, 52-60.

Lienard, D., Lejeune, F. J., & Ewalenko, P. (1992b). In transit metastases of malignant melanoma treated by high dose rTNF alpha in combination with interferon-gamma and melphalan in isolation perfusion. World J. Surg. 16, 234-240.

Lin, L. L., Wartmann, M., Lin, A. Y., Knopf, J. L., Seth, A., & Davis, R. J. (1993). $cPLA_2$ is phosphorylated and activated by MAP-kinase. Cell 72, 269-278.

Liu, A. Y., Miskovsky, E. P., Stanhope, P. E., & Siliciano, R. F. (1992). Production of transmembrane and secreted forms of tumor necrosis factor (TNF)-alpha by

HIV-1-specific $CD4^+$ cytolytic T lymphocyte clones. Evidence for a TNF-alpha-independent cytolytic mechanism. J. Immunol. 148, 3789-3798.

Liu, J., Mathias, S., Yang, Z. H., & Kolesnick, R. N. (1994). Renaturation and tumor necrosis factor-alpha stimulation of a 97-kDa ceramide-activated protein kinase. J. Biol. Chem. 269, 3047-3052.

Loetscher, H., Pan, Y. C., Lahm, H. W., Gentz, R., Brockhaus, M., Tabuchi, H., & Lesslauer, W. (1990). Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. Cell 61, 351-359.

Loetscher, H., Stueber, D., Banner, D., Mackay, F., & Lesslauer, W. (1993). Human tumor necrosis factor-alpha (TNF alpha) mutants with exclusive specificity for the 55-kDa or 75-kDa TNF receptors. J. Biol. Chem. 268, 26350-26357.

Lucas, R., Magez, S., De Leys, R., Fransen, L., Scheerlinck, J. P., Rampelberg, M., Sablon, E., & De Baetselier, P. (1994). Mapping the lectin-like activity of tumor necrosis factor. Science. 263, 814-817.

Luo, G., Niesel, D. W., Shaban, R. A., Grimm, E. A., & Klimpel, G. R. (1993). Tumor necrosis factor alpha binding to bacteria: Evidence for a high-affinity receptor and alteration of bacterial virulence properties. Infect. Immun. 61, 830-835.

MacNaul, K. L., Chartrain, N., Lark, M., Tocci, M. J., & Hutchinson, N. I. (1990). Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. Synergistic effects of interleukin-1 and tumor necrosis factor-alpha on stromelysin expression. J. Biol. Chem. 265, 17238-17245.

Maimone, D., Gregory, S., Arnason, B. G., & Reder, A. T. (1991). Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. J. Neuroimmunol. 32, 67-74.

Maini, R. N., Brennan, F. M., Williams, R., Chu, C. Q., Cope, A. P., Gibbons, D., Elliott, M., & Feldmann, M. (1993). TNF-alpha in rheumatoid arthritis and prospects of anti-TNF therapy. Clin. Exp. Rheumatol. 11, 173-175.

Manda, T., Shimomura, K., Mukumoto, S., Kobayashi, K., Mizoto, T., & Hirai, O. (1987). Recombinant human tumor necrosis factor-alpha: Evidence of an indirect mode of antitumor activity. Cancer Res. 47, 3707-3711.

Männel, D. N., Moore, R. N., & Mergenhager, S. E. (1980). Macrophages as a major source of tumoricidal activity (tumor necrotizing factor). Infect. Immunol. 30, 532-539.

Matsuyama, T., Hamamoto, Y., Soma, G., Mizuno, D., Yamamoto, N., & Kobayashi, N. (1989). Cytocidal effect of tumor necrosis factor on cells chronically infected with human immunodeficiency virus (HIV): Enhancement of HIV replication. J. Virol. 63, 2504-2509.

Matthews, N., Neale, M. L., Jackson, S. K., & Stark, J. M. (1987). Tumour cell killing by tumour necrosis factor: Inhibition by anaerobic conditions, free-radical scavengers and inhibitors of arachidonate metabolism. Immunology 62, 153-155.

McGeehan, G. M., Becherer, J. D., Bast, R. C., Boyer, C. M., Champion, B., Connolly, K. M., Conway, J. G., Furdon, P., Karp, S., Kidao, S., McElroy, A. B., Nichols, J., Pryzwansky, K. M., Schoenen, F., Sekut, L., Truesdale, A., Verghese, M., Warner, J., & Ways, J. P. (1994). Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor. Nature 370, 558-561.

Meager, A., Leung, H., & Woolley, J. (1989). Assays for tumour necrosis factor and related cytokines. J. Immunol. Meth. 116, 1-17.

Meichle, A., Schütze, S., Hensel, G., Brunsing, D., & Krönke, M. (1990). Protein kinase C-independent activation of nuclear factor kappa B by tumor necrosis factor. J. Biol. Chem. 265, 8339-8343.

Merrill, J. E., Strom, S. R., Ellison, G. W., & Myers, L. W. (1989). *In vitro* study of mediators of inflammation in multiple sclerosis. J. Clin. Immunoly. 9, 84-96.

Mestan, J., Brockhaus, M., Kirchner, H., & Jacobsen, H. (1988). Antiviral activity of tumour necrosis factor. Synergism with interferons and induction of oligo-2',5'-adenylate synthetase. J. Gen. Virol. 69, 3113-3120.

Michie, H. R., Manogue, K. R., Spriggs, D. R., Revhaug, A., O'Dwyer, S., Dinarello, C. A., Cerami, A., Wolff, S. M., & Wilmore, D. W. (1988). Detection of circulating tumor necrosis factor after endotoxin administration. N. Engl. J. Med. 318, 1481-1486.

Michihiko, S., Yamamoto, N., Shinozaki, F., Shimada, K., Soma, G., & Kobayashi, N. (1989). Augmentation of *in vitro* HIV replication in peripheral blood mononuclear cells of AIDS and ARC patients by tumour necrosis factor. Lancet 1, 1206-1207.

Mirzabekov, T., Baldwin, R. L., Kagan, B. L., & Wisnieski, B. J. (1994). The pH-dependent step of TNFα and TNF-β channel activity is bilayer insertion. Eur. Cytokine Netw. 5, 87.

Mohler, K. M., Torrance, D. S., Smith, C. A., Goodwin, R. G., Stremler, K. E., Fung, V. P., Madani, H., & Widmer, M. B. (1993). Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J. Immunol. 151, 1548-1561.

Mohler, K. M., Sleath, P. R., Fitzner, J. N., Cerretti, D. P., Alderson, M., Kerwar, S. S., Torrance, D. S., Ottenevans, C., Greenstreet, T., Weerawarna, K., Kronheim, S. R., Petersen, M., Gerhart, M., Kozlosky, C. J., March, C. J., & Black, R. A. (1994). Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. Nature 370, 218-220.

Moreira, A. L., Sampaio, E. P., Zmuidzinas, A., Frindt, P., Smith, K. A., & Kaplan, G. (1993). Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. J. Exp. Med. 177, 1675-1680.

Muchmore, A., Decker, J., Shaw, A., & Wingfield, P. (1990). Evidence that high mannose glycopeptides are able to functionally interact with recombinant tumor necrosis factor and recombinant interleukin 1. Cancer Res. 50, 6285-6290.

Müller, R., Marmenout, A., & Fiers, W. (1986). Synthesis and maturation of recombinant human tumor necrosis factor in eukaryotic systems. FEBS Lett. 197, 99-104.

Müller, G., Ajoub, M., Storz, P., Renneke, J., Fabbro, D., & Pfizenmaier, K. (1995). PKC ζ is a molecular switch in signal transduction of TNF-α, bifunctionally regulated by ceramide and arachidonic acid. EMBO J. 14, 1961-1969.

Mundy, G. R. (1989). Hypercalcemic factors other than parathyroid hormone-related protein. Endocrinol. Metab. Clin. North Am. 18, 795-806.

Mundy, G. R., Roodman, G. D., Bonewald, L. F., Yoneda, T., & Sabatini, M. (1992). Effects of TNF and lymphotoxin on bone cells. Immunol. Ser. 56, 483-498.

Munker, R., Gasson, J., Ogawa, M., & Koeffler, H. P. (1986). Recombinant human TNF induces production of granulocyte-monocyte colony-stimulating factor. Nature 323, 79-82.

Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O'Rourke, K., Shevchenko, A., Ni., J. Scaffidi, C., Bretz, J. D., Zhang, M., Gentz, R., Mann, M., Krammer, P. H., Peter M. E., & Dixit, V. M. (1996). FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85, 817-827.

Nagata, S. (1994). Mutations in the Fas antigen gene in *lpr* mice. Semin. Immunol. 6, 3-8.

Nakane, A., Minagawa, T., & Kato, K. (1988). Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. Infect. Immun. 56, 2563-2569.

Nakane, A., Numata, A., & Minagawa, T. (1992). Endogenous tumor necrosis factor, interleukin-6, and gamma interferon levels during *Listeria monocytogenes* infection in mice. Infect. Immun. 60, 523-528.

Naotunne, T. S., Karunaweera, N. D., Del Giudice, G., Kularatne, M. U., Grau, G. E., Carter, R., & Mendis, K. N. (1991). Cytokines kill malaria parasites during infection crisis: Extracellular complementary factors are essential. J. Exp. Med. 173, 523-529.

Nargi, F. E., & Yang, T. J. (1993). Optimization of the L-M cell bioassay for quantitating tumor necrosis factor alpha in serum and plasma. J. Immunol. Meth. 159, 81-91.

Nawroth, P. P., Bank, I., Handley, D., Cassimeris, J., Chess, L., & Stern, D. (1986). Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J. Exp. Med. 163, 1363-1375.

Nedwin, G. E., Naylor, S. L., Sakaguchi, A. Y., Smith, D., Jarrett-Nedwin, J., Pennica, D., Goeddel, D. V., & Gray, P. W. (1985). Human lymphotoxin and tumor necrosis factor genes: Structure, homology and chromosomal localization. Nucleic Acids Res. 13, 6361-6373.

Nemenoff, R. A., Winitz, S., Qian, N. X., Van Putten, V., Johnson, G. L., & Heasley, L. E. (1993). Phosphorylation and activation of a high molecular weight form of phospholipase A_2 by p42-microtubule-associated protein 2 kinase and protein kinase C. J. Biol. Chem. 268, 1960-1964.

Nokta, M., Matzke, D., Jennings, M., Schlick, E., Nadler, P. I., & Pollard, R. (1991). *In vivo* administration of tumor necrosis factor-alpha is associated with antiviral activity in human peripheral mononuclear cells. Proc. Soc. Exp. Biol. Med. 197, 144-149.

Nussler, A., Pied, S., Goma, J., Renia, L., Miltgen, F., Grau, G. E., & Mazier, D. (1991). TNF inhibits malaria hepatic stages *in vitro* via synthesis of IL-6. Int. Immunol. 3, 317-321.

Obeid, L. M., Linardic, C. M., Karolak, L. A., & Hannun, Y. A. (1993). Programmed cell death induced by ceramide. Science 259, 1769-1771.

Oehm, A., Behrmann, I., Falk, W., Pawlita, M., Maier, G., Klas, C., Li-Weber, M., Richards, S., Dhein, J., Trauth, B. C., Ponsting, H., & Krammer, P. H. (1992). Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen. J. Biol. Chem. 267, 10709-10715.

Ohno, K., Nakano, T., Matsumoto, Y., Watari, T., Goitsuka, R., Nakayama, H., Tsujimoto, H., & Hasegawa, A. (1993). Apoptosis induced by tumor necrosis factor in cells chronically infected with feline immunodeficiency virus. J. Virol. 67, 2429-2433.

Orosz, P., Echtenacher, B., Falk, W., Ruschoff, J., Weber, D., & Männel, D. N. (1993). Enhancement of experimental metastasis by tumor necrosis factor. J. Exp. Med. 177, 1391-1398.

Osborn, L., Kunkel, S., & Nabel, G. J. (1989a). Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Proc. Natl. Acad. Sci. USA 86, 2336-2340.

Osborn, L., Hession, C., Tizard, R., Vassallo, C., Luhowskyj, S., Chi-Rosso, G., & Lobb, R. (1989b). Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell. 59, 1203-1211.

Palladino, M. A., Shalaby, M. R., Kramer, S. M., Ferraiolo, B. L., Baughman, R. A., Deleo, A. B., Crase, D., Marafino, B., Aggarwal, B. B., & Figari, I. S. (1987) Characterization of the antitumor activities of human tumor necrosis factor-alpha and the comparison with other cytokines: Induction of tumor-specific immunity. J. Immunol. 138, 4023-4032.

Palombella, V. J., Yamashiro, D. J., Maxfield F. R., Decker, S. J., & Vilcek, J. (1987). Tumor necrosis factor increases the number of epidermal growth factor receptors on human fibroblasts. J. Biol. Chem. 262, 1950-1954.

Paterson, P. Y. (1977). Autoimmune neurological diseases: experimental animal systems and implications for multiple sclerosis. In: *Autoimmunity: Genetic, Immunologic Virologic, and Clinical Aspects* (Talel, N., Ed.). Academic Press, New York, pp. 643-692.

Peck, R., Brockhaus, M., & Frey, J. R. (1989). Cell surface tumor necrosis factor (TNF) accounts for monocyte- and lymphocyte-mediated killing of TNF-resistant target cells. Cell. Immunol. 122, 1-10.

Peck, R., Brockhaus, M., & Frey, J. R. (1991). The principal tumor necrosis factor receptor in monocyte cytotoxicity is on the effector cell, not on the target cell. Cell. Immunol. 132, 308-318.

Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B., & Goeddel, D. V. (1984). Human tumor necrosis factor: Precursor structure, expression and homology to lymphotoxin. Nature 312, 724-729.

Perez, C., Albert, I., DeFay, K., Zachariades, N., Gooding, L., & Kriegler, M. (1990). A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. Cell 63, 251-258.

Peters, P. M., Ortaldo, J. R., Shalaby, M. R., Svedersky, L. P., Nedwin, G. E., Bringman, T. S., Hass, P. E., Aggarwal, B. B., Herberman, R. B., & Goeddel, D. V. (1986). Natural killer-sensitive targets stimulate production of TNFα but not TNF-beta (lymphotoxin) by highly purified human peripheral blood large granular lymphocytes. J. Immunol. 137, 2592-2598.

Peterson, P. K., Gekker, G., Chao, C. C., Hu, S. X., Edelman, C., Balfour, H. H., Jr., & Verhoef, J. (1992). Human cytomegalovirus-stimulated peripheral blood mononuclear cells induce HIV-1 replication via a tumor necrosis factor-alpha-mediated mechanism. J. Clin. Invest. 89, 574-580.

Pfeffer, K., Matsuyama, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Krönke, M., & Mak, T. W. (1993). Mice deficient for the 55kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. Cell 73, 457-467.

Pfizenmaier, K., Scheurich, P., Schlüter, C., & Krönke, M. (1987). Tumor necrosis factor enhances HLA-A,B,C and HLA-DR gene expression in human tumor cells. J. Immunol. 138, 975-980.

Pfizenmaier, K., A. Himmler, S. Schütze, P. Scheurich, & M. Krönke. (1992). TNF receptors and TNF signal transduction. In: *Tumor Necrosis Factors: The Molecules and Their Emerging Role in Medicine* (Beutler, B., Ed.). Raven Press, New York, pp. 439-472.

Philip, R., & Epstein, L. B. (1986). Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, gamma-interferon and interleukin-1. Nature 323, 86-89.

Piguet, P. F., Grau, G. E., Vesin, C., Loetscher, H., Gentz, R., & Lesslauer, W. (1992). Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. Immunology 77, 510-514.

Pisa, P., Gennene, M., Soder, O., Ottenhoff, T., Hansson, M., & Kiessling, R. (1990). Serum tumor necrosis factor levels and disease dissemination in leprosy and leishmaniasis. J. Infect. Dis. 161, 988-991.

Poli, G., Kinter, A., Justement, J. S., Kehrl, J. H., Bressler, P., Stanley, S., & Fauci, A. S. (1990). Tumor necrosis factor alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression. Proc. Natl. Acad. Sci. USA 87, 782-785.

Porteu, F., & Hieblot, C. (1994). Tumor necrosis factor induces a selective shedding of its p75 receptor from human neutrophils. J. Biol. Chem. 269, 2834-2840.

Porteu, F., Brockhaus, M., Wallach, D., Engelmann, H., & Nathan, C. F. (1991). Human neutrophil elastase releases a ligand-binding fragment from the 75-kDa tumor necrosis factor (TNF) receptor. Comparison with the proteolytic activity responsible for shedding of TNF receptors from stimulated neutrophils. J. Biol. Chem. 266, 18846-18853.

Powell, M. B., Mitchell, D., Lederman, J., Buckmeier, J., Zamvil, S. S., Graham, M., Ruddle, N. H., & Steinman, L. (1990). Lymphotoxin and tumor necrosis factor-alpha production by myelin basic protein-specific T cell clones correlates with encephalitogenicity. Int. Immunol. 2, 539-544.

Raines, M. A., Kolesnick, R. N., & Golde, D. W. (1993). Sphingomyelinase and ceramide activate mitogen-activated protein kinase in myeloid HL-60 cells. J. Biol. Chem. 268, 14572-14575.

Ranges, G. E., Zlotnik, A., Espevik, T., Dinarello, C. A., Cerami, A., & Palladino, M. A., Jr. (1988). Tumor necrosis factor alpha/cachectin is a growth factor for thymocytes. Synergistic interactions with other cytokines. J. Exp. Med. 167, 1472-1478.

Ratner, A., & Clark, W. R. (1993). Role of TNF-alpha in $CD8^+$ cytotoxic T-lymphocyte-mediated lysis. J. Immunol. 150, 4303-4314.

Remick, D. G., Kunkel, R. G., Larrick, J. W., & Kunkel, S. L. (1987). Acute *in vivo* effects of human recombinant tumor necrosis factor. Lab. Invest. 56, 583-590.

Robbins, D. S., Shirazi, Y., Drysdale, B. E., Liebermann, A., Shin, H. S., & Shin, M. L. (1987). Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. J. Immunol. 139, 2593-2597.

Rodriguez, C., Roldan, E., Navas, G., & Brieva, J. A. (1993). Essential role of tumor necrosis factor-alpha in the differentiation of human tonsil *in vivo* induced B cells capable of spontaneous and high-rate immunoglobulin secretion. Eur. J. Immunol. 23, 1160-1164.

Rook, G. A., Attiyah, R. A., & Foley, N. (1989). The role of cytokines in the immunopathology of tuberculosis, and the regulation of agalactosyl IgG. Lymphokine Res. 8, 323-328.

Rothe, J., Lesslauer, W., Loetscher, H., Lang, Y., Koebel, P., Kontgen, F., Althage, A., Zinkernagel, R., Steinmetz, M., & Bluethmann, H. (1993). Mice lacking the tumour necrosis factor receptor-1 are resistant to TNF-mediated toxicity but highly susceptible to infection by listeria monocytogenes. Nature 364, 798-802.

Rothe, M., Wong, S. C., Henzel, W. J., & Goeddel, D. V. (1994). A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. Cell 78, 681-692.

Rothe, M., Sarma, V., Dixit, V. M., & Goeddel, D. V. (1995a). TRAF2-mediated activation of BF-κB by TNF receptor 2 and CD40. Science 269, 1424-1427.

Rothe, M., Pan, M.-G., Henzel, W. J., Ayres, T. M., & Goeddel, D. (1995b). The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 83, 1243-1252.

Rothe, M., Xiong, J., Shu, H.-B., Williamson, K., Goddard, A., & Goeddel, D. (1996). I-TRAF is a novel TRAF-interacting protein that regulates TRAF-mediated signal transduction. Proc. Natl. Acad. Sci. USA 93, 8241-8246.

Rothstein, J. L., & Schreiber, H. (1988). Synergy between tumor necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. Proc. Natl. Acad. Sci. USA 85, 607-611.

Rouzer, C., & Cerami, A. (1980). Hypertriglyceridemia associated with *Trypanosoma brucei* infection in rabbits: Role of defective triglyceride removal. Mol. Biochem. Parasitol. 2, 31-37.

Rubin, B. Y., Anderson, S. L., Sullivan, S. A., Williamson, B. D., Carswell, E. A., & Old, L. J. (1986). Nonhematopoietic cells selected for resistance to tumor necrosis factor produce tumor necrosis factor. J. Exp. Med. 164, 1350-1355.

Ruddle, N. H., Bergman, C. M., McGrath, K. M., Lingenheld, E. G., Grunnet, M. L., Padula, S. J., & Clark, R. B. (1990). An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J. Exp. Med. 172, 1193-1200.

Ruggiero, V., Johnston, S., & Baglioni, C. (1987). Protection from tumor necrosis factor cytotoxicity by protease inhibitors. Cell. Immunol. 107, 317-325.

Santambrogio, L., Hochwald, G. M., Saxena, B., Leu, C. H., Martz, J. E., Carlino, J. A., Ruddle, N. H., Palladino, M. A., Gold, L. I., & Thorbecke, G. J. (1993). Studies on the mechanisms by which transforming growth factor-beta (TGF-beta) protects against allergic encephalomyelitis. Antagonism between TGF-beta and tumor necrosis factor. J. Immunol. 151, 1116-1127.

Sariban, E., Imamura, K., Luebbers, R., & Kufe, D. (1988). Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. J. Clin. Invest. 81, 1506-1510.

Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H., Gatanaga, T., Granger, G. A., Lentz, R., Raab, H., Kohr, W. J., & Groeddel, D. J. (1990).

Molecular cloning and expression of a receptor for human tumor necrosis factor. Cell 61, 361-370.

Scheurich, P., Thoma, B., Ücer, U., & Pfizenmaier, K. (1987). Immunoregulatory activity of recombinant human tumor necrosis factor (TNFα: Induction of TNF receptors on human T cells and TNF-α-mediated enhancement of T cell responses. J. Immunol. 138, 1786-1790.

Scheurich, P., Köbrich, G., & Pfizenmaier, K. (1989). Antagonistic control of tumor necrosis factor receptors by protein kinases A and C. Enhancement of TNF receptor synthesis by protein kinase A and transmodulation of receptors by protein kinase C. J. Exp. Med. 170, 947-958.

Scheurich, P., Grell, M., Meager, A., & Pfizenmaier, K. (1993). Agonistic and antagonistic antibodies as a tool to study the functional role of human tumor necrosis factor receptors. In: *Tumor Necrosis Factor: Molecular and Cellular Biology and Clinical Relevance* (Fiers, W., & Buurman, W. A., Eds.). Karger, Basel, Switzerland, pp. 52-57.

Schmid, E., Müller, T. H., Budzinski, R.-M., Pfizenmaier, K., & Binder, K. (1995). Lymphocyte adhesion to human endothelial cells induces tissue factor expression via a juxtacrine pathway. Thromb. Haemost. 73, 421-428.

Schulze-Osthoff, K., Bakker, A. C., Vanhaesebroeck, B., Beyaert, R., Jacob, W. A., & Fiers, W. (1992). Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. J. Biol. Chem. 267, 5317-5323.

Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G., & Fiers, W. (1993). Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J. 12, 3095-3104.

Schütze, S., Nottrott, S., Pfizenmaier, K., & Krönke, M. (1990). Tumor necrosis factor signal transduction. Cell-type-specific activation and translocation of protein kinase C. J. Immunol. 144, 2604-2608.

Schütze, S., Berkovic, D., Tomsing, O., Unger, C., & Krönke, M. (1991). Tumor necrosis factor induces rapid production of 1'2'diacylglycerol by a phosphatidylcholine-specific phospholipase C. J. Exp. Med. 174, 975-988.

Schütze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K., & Krönke, M. (1992). TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. Cell 71, 765-767.

Schwarz, H., Tuckwell, J., & Lotz, M. (1993). A receptor induced by lymphocyte activation (Ila)—A new member of the human nerve-growth-factor tumor-necrosis-factor receptor family. Gene 134, 295-298.

Seckinger, P., Zhang, J. H., Hauptmann, B., & Dayer, J. M. (1990). Characterization of a tumor necrosis factor alpha (TNF-alpha) inhibitor: Evidence of immunological cross-reactivity with the TNF receptor. Proc. Natl. Acad. Sci. USA 87, 5188-5192.

Selmaj, K. W., & Raine, C. S. (1988). TNF mediated myelin and oligodendrocyte damage *in vitro*. Ann. Neurol. 23, 339-346.

Shalaby, M. R., Sundan, A., Loetscher, H., Brockhaus, M., Lesslauer, W., & Espevik, T. (1990). Binding and regulation of cellular functions by monoclonal antibodies against human tumor necrosis factor receptors. J. Exp. Med. 172, 1517-1520.

Sherblom, A. P., Decker, J. M., & Muchmore, A. V. (1988). The lectin-like interaction between recombinant tumor necrosis factor and uromodulin. J. Biol. Chem. 263, 5418-5424.

Smith, C. A., Davis, T., Anderson, D., Solam, L., Beckmann, M. P., Jerzy, R., Dower, S. K., Cosman, D., & Goodwin, R. G. (1990). A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 248, 1019-1023.

Smith, M. R., Munger, W. E., Kung, H. F., Takacs, L., & Durum, S. K. (1990). Direct evidence for an intracellular role for tumor necrosis factor-alpha 1. Microinjection of tumor necrosis factor kills target cells. J. Immunol. 144, 162-169.

Smith, C. A., Davis, T., Wignall, J. M., Din, W. S., Farrah, T., Upton, C., McFadden, G., & Goodwin, R. G. (1991). T2 open reading frame from the *Shope fibroma* virus encodes a soluble form of the TNF receptor. Biochem. Biophys. Res. Commun. 176, 335-342.

Smith, C. A., Farrah, T., & Goodwin, R. G. (1994). The TNF receptor superfamily of cellular and viral proteins—activation, costimulation, and death. Cell 76, 959-962.

Smith, R. A., & Baglioni, C. (1987). The active form of tumor necrosis factor is a trimer. J. Biol. Chem. 262, 6951-6956.

Smyth, M. J., & Ortaldo, J. R. (1993). Mechanisms of cytotoxicity used by human peripheral blood CD4+ and CD8+ T cell subsets. The role of granule exocytosis. J. Immunol. 151, 740-747.

Song, H.Y., Rothe, M., & Goeddel, D. V., (1996). The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-κB activation. Proc. Natl. Acad. Sci. USA 93, 6721-6725.

Suffys, P., Beyaert, R., Van Roy, F., & Fiers, W. (1987). Reduced tumour necrosis factor-induced cytotoxicity by inhibitors of the arachidonic acid metabolism. Biochem. Biophys. Res. Commun. 149, 735-743.

Suffys, P., Beyaert, R., Van Roy, F., & Fiers, W. (1988). Involvement of a serine protease in tumour-necrosis-factor-mediated cytotoxicity. Eur. J. Biochem. 178, 257-265.

Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, I. S., Palladino, M. A., & Shepard, H. M. (1985). Recombinant human tumor necrosis factor-α: Effects on proliferation of normal and transformed cells *in vitro*. Science 230, 943-945.

Sung, S. S., Jung, L. K., Walters, J. A., Chen, W., Wang, C. Y., & Fu, S. M. (1988a). Production of tumor necrosis factor/cachectin by human B cell lines and tonsillar B cells. J. Exp. Med. 168, 1539-1551.

Sung, S. S., Bjorndahl, J. M., Wang, C. Y., Kao, H. T., & Fu, S. M. (1988b). Production of tumor necrosis factor/cachectin by human T cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody. J. Exp. Med. 167, 937-953.

Sypek, J. P., & Wyler, D. J. (1991). Antileishmanial defense in macrophages triggered by tumor necrosis factor expressed on $CD4^+$ T lymphocyte plasma membrane. J. Exp. Med. 174, 755-759.

Sypek, J. P., Jacobson, S., Vorys, A., & Wyler, D. J. (1993). Comparison of gamma interferon, tumor necrosis factor, and direct cell contact in activation of antimycobacterial defense in murine macrophages. Infect. Immun. 61, 3901-3906.

Talmadge, J. E. (1992). Synergy in the toxicity of cytokines: Preclinical studies. Int. J. Immunopharmacol. 14, 383-390.

Tartaglia, L. A., Weber, R. F., Figari, I. S., Reynolds, C., Palladino, M. A., Jr., & Goeddel, D. V. (1991). The two different receptors for tumor necrosis factor mediate distinct cellular responses. Proc. Natl. Acad. Sci. USA 88, 9292-9296.

Tartaglia, L. A., Ayres, T. M., Wong, G. H., & Goeddel, D. V. (1993a). A novel domain within the 55 kd TNF receptor signals cell death. Cell 74, 845-853.
Tartaglia, L. A., Goeddel, D. V., Reynolds, C., Figari, I. S., Weber, R. F., Fendly, B. M., & Palladino, M. A., Jr. (1993b). Stimulation of human T-cell proliferation by specific activation of the 75-kDa tumor necrosis factor receptor. J. Immunol. 151, 4637-4641.
Tartaglia, L. A., Pennica, D., & Goeddel, D. V. (1993c). Ligand passing: The 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J. Biol. Chem. 268, 18542-18548.
Teng, M. N., Park, B. H., Koeppen, H. K., Tracey, K. J., Fendly, B. M., & Schreiber, H. (1991). Long-term inhibition of tumor growth by tumor necrosis factor in the absence of cachexia or T-cell immunity. Proc. Natl. Acad. Sci. USA 88, 3535-3539.
Thanhauser, A., Reiling, N., Bohle, A., Toellner, K. M., Duchrow, M., Scheel, D., Schluter, C., Ernst, M., Flad, H. D., & Ulmer, A. J. (1993). Pentoxifylline: a potent inhibitor of IL-2 and IFN-gamma biosynthesis and BCG-induced cytotoxicity. Immunology 80, 151-156.
Thoma, B., Grell, M., Pfizenmaier, K., & Scheurich, P. (1990). Identification of a 60-kD tumor necrosis factor (TNF) receptor as the major signal transducing component in TNF responses. J. Exp. Med. 172, 1019-1023.
Tiegs, G., Barsig, J., Matiba, B., Uhlig, S., & Wendel, A. (1994). Potentiation by granulocyte macrophage colony-stimulating factor of lipopolysaccharide toxicity in mice. J. Clin. Invest. 93, 2616-2622.
Tite, J. P. (1990). Evidence of a role for TNF-alpha in cytolysis by $CD4^+$, class II MHC-restricted cytotoxic T cells. Immunology 71, 208-212.
Tracey, K. J. (1991). Tumor necrosis factor (cachectin) in the biology of septic shock syndrome. Circ. Shock 35, 123-128.
Tracey, K. J., & Cerami, A. (1993). Tumor necrosis factor, other cytokines and disease. Annu. Rev. Cell Biol. 9, 317-343.
Tracey, K. J., Beutler, B., Lowry, S. F., Merryweather, J., Wolpe, S., Milsark, I. W., Hariri, R. J., Fahey, T. J., III, Zentella, A., & Alberts, J. D. (1986). Shock and tissue injury induced by recombinant human cachectin. Science 234, 470-474.
Tracey, K. J., Fong, Y., Hesse, D. G., Manogue, K. R., Lee, A. T., Kuo, G. C., Lowry, S. F., & Cerami, A. (1987). Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature. 330, 662-664.
Trefzer, U., Brockhaus, M., Loetscher, H., Parlow, F., Budnik, A., Grewe, M., Christoph, H., Kapp, A., Schopf, E., Luger, T. A., & Krutmann, J. (1993). The 55-kD tumor necrosis factor receptor on human keratinocytes is regulated by tumor necrosis factor-alpha and by ultraviolet B radiation. J. Clin. Invest. 92, 462-470.
Tsujimoto, M., Yip, Y. K., & Vilcek, J. (1986). Interferon-gamma enhances expression of cellular receptors for tumor necrosis factor. J. Immunol. 136, 2441-2444.
Ücer, U., Scheurich, P., Bartsch, H., Berkovic, D., Ertel, C., & Pfizenmaier, K. (1987). Specific membrane receptors for human interferon-gamma (IFN-gamma). Behring Inst. Mitt. 81, 88-97.
Ueno, Y., Suzuki, H., Kobayashi, K., Miura, M., & Toyota, T. (1990). Production of tumor necrosis factor alpha and interleukin 1 beta by peripheral blood mononuclear cells from chronic hepatitis type C patients during interferon therapy. Tohoku J. Exp. Med. 161, 157-158.

Unglaub, R., Maxeiner, B., Thoma, B., Pfizenmaier, K., & Scheurich, P. (1987). Downregulation of tumor necrosis factor (TNF) sensitivity via modulation of TNF binding capacity by protein kinase C activators. J. Exp. Med. 166, 1788-1797.

Valone, S. E., Rich, E. A., Wallis, R. S., & Ellner, J. J. (1988). Expression of tumor necrosis factor *in vitro* by human mononuclear phagocytes stimulated with whole Mycobacterium bovis BCG and mycobacterial antigens. Infect. Immun. 56, 3313-3315.

Van Arsdale, T. L., & Ware, C. F. (1994). TNF receptor signal transduction—Ligand-dependent stimulation of a serine protein kinase activity associated with (CD120a) $TNFR_{60}$. J. Immunol. 153, 3043-3050.

Van Lint, J., Agostinis, P., Vandevoorde, V., Haegeman, G., Fiers, W., Merlevede, W., & Vandenheede, J. R. (1992). Tumor necrosis factor stimulates multiple serine/threonine protein kinases in Swiss 3T3 and L929 cells. Implication of casein kinase-2 and extracellular signal-regulated kinases in the tumor necrosis factor signal transduction pathway. J. Biol. Chem. 267, 25916-25921.

Van Ostade, X., Vandenabeele, P., Everaerdt, B., Loetscher, H., Gentz, R., Brockhaus, M., Lesslauer, W., Tavernier, J., Brouckaert, P., & Fiers, W. (1993). Human TNF mutants with selective activity on the p55 Receptor. Nature 361, 266-269.

Van Ostade, X., Vandenabeele, P., Tavernier, J., & Fiers, W. (1994). Human tumor necrosis factor mutants with preferential binding to and activity on either the R55 or R75 receptor. Eur. J. Biochem. 220, 771-779.

Van Zee, K. J., Stackpole, S. A., Montegut, W. J., Rogy, M. A., Calvano, S. E., Hsu, K. C., Chao, M., Meschter, C. L., Loetscher, H., Stuber, D., Ettlin, U., Wipf, B., Lesslauer, W., Lowry, S. F., & Moldawer, L. L. (1994). A human tumor necrosis factor (TNF) alpha mutant that binds exclusively to the p55 TNF receptor produces toxicity in the baboon. J. Exp. Med. 179, 1185-1191.

Van Zee, K. J., Moldawer, L. L., Oldenburg, H. S. A., Thompson, W. A., Stackpole, S. A., Montegut, W. J., Rogy, M. A., Meschter, C., Gallati, H., Schiller, C.-D., Richter, W. F., Loetscher, H., Ashkenazi, A., Chamow, S. M., Wurm, F., Calvano, S. E., Lowry, S. F., & Lesslauer, W. (1996). Protection against *Escherichia coli* Bacteremia in baboons (*Papio anubis*) by pretreatment with a 55-kDa TNF receptor (CD120a)-lg fusion protein, Ro 45-2081. J. Immunol. 156, 2221-2230.

Voskuhl, R. R., Martin, R., Bergman, C., Dalal, M., Ruddle, N. H., & McFarland, H. F. (1993). T helper 1 (Thl) functional phenotype of human myelin basic protein-specific T lymphocytes. Autoimmunity 15, 137-143.

Waage, A., Halstensen, A., & Espevik, T. (1987). Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 355-357.

Waage, A., & Espevik, T. (1988). Interleukin 1 potentiates the lethal effect of tumor necrosis factor alpha/cachectin in mice. J. Exp. Med. 167, 1987-1992.

Waage, A., Brandtzaeg, P., Halstensen, A., Kierulf, P., & Espevik, T. (1989). The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J. Exp. Med. 169, 333-338.

Wallach, D., Holtmann, H., Engelmann, H., & Nophar, Y. (1988). Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. J. Immunol. 140, 2994-2999.

Wang, A. M., Creasey, A. A., Ladner, M. B., Lin, L. S., Strickler, J., Van Arsdell, J. N., Yamamoto, R., & Mark, D. F. (1985). Molecular cloning of the complementary DNA for human tumor necrosis factor. Science. 228, 149-154.

Warren, M. K., & Ralph, P. (1986). Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor, and colony stimulating activity. J. Immunol. 137, 2281-2285.

Webb, G. C., & Chaplin, D. D. (1990). Genetic variability at the human tumor necrosis factor loci. J. Immunol. 145, 1278-1285.

Wiegmann, K., Schütze, S., Kampen, E., Himmler, A., Machleidt T., & Krönke, M. (1992). Human 55-kDa receptor for tumor necrosis factor coupled to signal transduction cascades. J. Biol. Chem. 267, 17997-18001.

Wiegmann, K., Schütze, S., Machleidt, T., Witte, D., & Krönke, M. (1994). Functional dichotomy of neutral and acidic sphingomyelinases in tumor necrosis factor signaling. Cell 78, 1005-1015.

Wiley, S. R., Schooly, K., Smolak, P. J., Din, W. S., Huang, C.-P., Nicholl, J. K., Sutherland, G. R., Smith, T. D., Rauch, C., Smith, C. A., & Goodwin, R. G. (1995). Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3, 673-682.

Williams, R. O., Mason, L. J., Feldmann, M., & Maini, R. N. (1994). Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. Proc. Natl. Acad. Sci. USA 91, 2762-2766.

Wilson, A. G., di Giovine, F. S., Blakemore, A. I., & Duff, G. W. (1992). Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum. Mol. Genet. 1, 353.

Wilson, A. G., de Vries, N., Pociot, F., di Giovine, F. S., van der Putte, L. B., & Duff, G. W. (1993). An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. J. Exp. Med. 177, 557-560.

Winzen, R., Wallach, D., Engelmann, H., Nophar, Y., Brakebusch, C., Kemper, O., Resch, K., & Holtmann, H. (1992). Selective decrease in cell surface expression and mRNA level of the 55-kDa tumor necrosis factor receptor during differentiation of HL-60 cells into macrophage-like but not granulocyte-like cells. J. Immunol. 148, 3454-3460.

Winzen, R., Wallach, D., Kemper, O., Resch, K., & Holtmann, H. (1993). Selective up-regulation of the 75-kDa tumor necrosis factor (TNF) receptor and its mRNA by TNF and IL-1. J. Immunol. 150, 4346-4353.

Wisniewski, H. G., Maier, R., Lotz, M., Lee, S., Klampfer, L., Lee, T. H., & Vilcek, J. (1993). TSG-6: A TNF-, IL-1-, and LPS-inducible secreted glycoprotein associated with arthritis. J. Immunol. 151, 6593-6601.

Wong, G. H., & Goeddel, D. V. (1988). Induction of manganous superoxide dismutase by tumor necrosis factor: Possible protective mechanism. Science. 242, 941-944.

Wong, G. H., Krowka, J. F., Stites, D. P., & Goeddel, D. V. (1988). *In vitro* anti-human immunodeficiency virus activities of tumor necrosis factor-alpha and interferon-gamma. J. Immunol. 140, 120-124.

Wong, G. H., Elwell, J. H., Oberley, L. W., & Goeddel, D. V. (1989). Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell. 58, 923-931.

Wong, G. H., Tartaglia, L. A., Lee, M. S., & Goeddel, D. V. (1992). Antiviral activity of tumor necrosis factor is signaled through the 55-kDa type I TNF receptor. J. Immunol. 149, 3350-3353.

Wood, P. R., & Clark, I. A. (1984). Macrophages from babesia and malaria infected mice are primed for monokine release. Parasite-Immunol. 6, 309-317.

Woods, K. M., & Chapes, S. K. (1993). Three distinct cell phenotypes of induced-TNF cytotoxicity and their relationship to apoptosis. J. Leuk. Biol. 53, 37-44.

Wooley, P. H., Dutcher, J., Widmer, M. B., & Gillis, S. (1993). Influence of a recombinant human soluble tumor necrosis factor receptor Fc fusion protein on type-II collagen-induced arthritis in mice. J. Immunol. 151, 6602-6607.

Wright, S. C., Kumar, P., Tam, A. W., Shen, N., Varma, M., & Larrick, J. W. (1992). Apoptosis and DNA fragmentation precede TNF-induced cytolysis in U937 cells. J. Cell. Biochem. 48, 344-355.

Yamauchi, N., Kuriyama, H., Watanabe, N., Neda, H., Maeda, M., & Niitsu, Y. (1989). Intracellular hydroxyl radical production induced by recombinant human tumor necrosis factor and its implication in the killing of tumor cells *in vitro*. Cancer Res. 49, 1671-1675.

Yang, Z., Costanzo, M., Golde, D. W., & Kolesnick, R. N. (1993). Tumor necrosis factor activation of the sphingomyelin pathway signals nuclear factor kappa B translocation in intact HL-60 cells. J. Biol. Chem. 268, 20520-20523.

Yokota, S., Geppert, T. D., & Lipsky, P. E. (1988). Enhancement of antigen- and mitogen-induced human T lymphocyte proliferation by tumor necrosis factor-alpha. J. Immunol. 140, 531-536.

Zechner, R., Newman, T. C., Sherry, B., Cerami, A., & Breslow, J. L. (1988). Recombinant human cachectin/tumor necrosis factor but not interleukin-1 alpha downregulates lipoprotein lipase gene expression at the transcriptional level in mouse 3T3-L1 adipocytes. Mol. Cell. Biol. 8, 2394-2401.

Zhang, Y. H., Lin, J. X., & Vilcek, J. (1990). Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a kappa B-like sequence. Mol. Cell. Biol. 10, 3818-3823.

LYMPHOTOXIN:

DEVELOPMENTS DURING THE LAST DECADE

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I. INTRODUCTION

Perhaps the earliest evidence for the existence of lymphotoxin-like activity was found more than 35 years ago when Govaerts (1960) described how lymphocytes from kidney-homografted dogs produced specific cytotoxic lesions in renal cultures obtained from the corresponding donors. This kind of indirect evidence continued to accumulate long before scientists were able to isolate and purify the responsible molecule. The next year, for example, Rosenau and Moon (1961) showed that sensitized lymphocytes from mice could destroy homologous L-cells (fibroblast) in culture and ruled out the involvement of complements and antibodies in this phenomenon. Ruddle and Waksman (1967) demonstrated that lymph node cells from inbred rats having delayed sensitivity to soluble proteins inhibit growth of syngeneic or allogeneic fibroblasts in the presence of specific antigens. That supernatants derived from lymphocytes of mixed but not syngeneic cultures are toxic to tumor cells was first reported by Granger and Williams (1968), who named the active agent lymphocyte cytotoxic factor. The production of a soluble cytotoxic factor by sensitized lymphocytes was also reported independently the same year by Ruddle and Waksman (1968). Subsequently, this factor was renamed lymphotoxin by Williams and Granger (1968). Although the

presence of the putative lymphotoxin- like molecules were also reported from humans, hamsters, and guinea pigs (Sawada et al., 1975; Fuhrer and Evans, 1982), its isolation and purification proved extremely difficult primarily because of its microheterogeneity and the minute quantities of the material produced by the lymphocytes (Aggarwal et al., 1984a).

It was not until 1983 that our laboratory succeeded in establishing the true identity of lymphotoxin by isolating and purifying homogeneous protein from several hundred liters of conditioned medium (Aggarwal et al., 1983, 1984b). We then succeeded in determining its complete structure (Aggarwal et al., 1985a) which led to its cDNA cloning (Gray et al., 1984). It was the isolation of lymphotoxin that led us to the identification, purification, and structure determination of a second cytotoxic factor, which was named tumor necrosis factor (TNF; Aggarwal et al., 1985b). Although antigenically distinct, the biological and structural determination of lymphotoxin and TNF revealed that the two factors are highly related and even bind to the same cell surface receptor (Pennica et al., 1984; Aggarwal et al., 1985c). These similarities led us to propose another name for lymphotoxin, TNF-β (Haas et al., 1985; Shalaby et al., 1985).

The present review is exclusively devoted to a discussion of lymphotoxin. For TNF, the reader should refer to a separate chapter in this volume. TNF will be discussed, but only in the context of lymphotoxin. Both lymphotoxin and TNF share a common bioassay which invloves lysis of actinomycin D-treated L-929 cells (Aggarwal et al., 1984b, 1985b), however, antigenically these two cytokines are quite distinct. Since it is now known that cells that produce lymphotoxin also produce TNF, it is not clear whether the molecule or its effects described in the literature before the structural elucidation of lymphotoxin are due to lymphotoxin or to lymphotoxin-related molecules (such as TNF). Also before 1984, no information was available to show antigenic differences between lymphotoxin and TNF. It is highly likely that cytotoxic molecule derived from B lymphocytes and referred to as TNF (Williamson et al., 1983) was in fact lymphotoxin. Some of the activities initially assigned to lymphotoxin were later reported to be due to another novel cytotoxic factor, leukoregulin (Ransom et al., 1985). Due to these types of uncertainties, we will restrict ourselves in reviewing the literature only to that appearing after 1984 when the true molecular identity of lymphotoxin was established.

II. CELLULAR PRODUCTION AND REGULATION OF LYMPHOTOXIN

Unlike TNF, the cellular production of lymphotoxin is restricted. This cytokine was originally isolated from the human B lymphoblastoid cell line RPMI 1788 (Aggarwal et al., 1984b; Aggarwal, 1985). Since then it has been reported that lymphotoxin is also produced by human tonsillar B lymphocytes, pre B cells, B cell lymphomas, and B lymphocyte cell lines (Sung et al., 1989; Laskov et al., 1990; Estrov et al., 1993), activated human T cells (TH1 and TH0 cells) and T cell lines (Stone-Wolff et al., 1984 , Kehrl et al., 1987; Ruddle and Schmid, 1987; Sung et al., 1988; Shimizu et al., 1989; Kronke et al., 1988; Mossman and Coffman, 1989), melanocytes and melanoma cell lines (Melani et al., 1993), and human myeloma cells (Garrett et al., 1987). Some transformed murine B cells also produce lymphotoxin (Laskov et al., 1990). Most of the T cells in the blood that produce lymphotoxin have been shown to be CD4+ cells (Andersson et al., 1989). Besides lymphocytes, TNF has been shown to be secreted by a wide variety of other cell types including monocytes, neutrophils, L-929 fibroblast, natural killer cells, vascular endothelial cells, mast cells, glial cells, astrocytes, kupffer cells, granulosa cells, and smooth muscle cells, however, none of these cells have been demonstrated to produce lymphotoxin. Moreover, several types of tumors including breast carcinoma, renal carcinoma, glioblastoma, and lymphoma have been reported to produce TNF but not lymphotoxin. Why the production of lymphotoxin, in general, is so restricted compared to TNF is not clear.

Even though some cells express both lymphotoxin and TNF, the regulation of their expression is quite independent (Pennica et al., 1984; Cuturi et al., 1987). Stimulation of peripheral blood lymphocytes with PMA and calcium ionophore leads mainly to the induction of TNF, whereas mitogenic stimulation leads primarily to expression of lymphotoxin (Cuturi et al., 1987). Similarly, endotoxin is a major inducer of TNF but not lymphotoxin (Muller-Alouf et al., 1994). The physiological inducers of lymphotoxin are T cell mitogens including IL-1, IL-2, IL-4, and IL-6 (Nedwin et al., 1985a; Kasid et al., 1990; Dett et al., 1991). When normal T cells are exposed to IL-2 and anti-T3, they produce both TNF and lymphotoxin (Steffen et al., 1988), but activation by staphylococcal enterotoxin (Fischer et al., 1990) or phorbol ester treatment leads only to lymphotoxin production (Aggarwal, 1985a; Jongeneel et al., 1989). The kinetics of lymphotoxin mRNA accumulation and protein

production has also been shown to differ from that of TNF (English et al., 1991). In T cells, TNF mRNA peaks at 6 h and lymphotoxin mRNA peaks at 18 h whereas in B cells the respective times are 24 h and 48 h (Sung et al., 1989). The half-life of the lymphotoxin mRNA was found to be eight to10-fold longer than that of TNF mRNA. Thus, lymphotoxin mRNA accumulates more slowly but persists much longer than that of TNF (English et al., 1991). The differential regulation of lymphotoxin and TNF is also evident from studies in which the treatment of human B lymphocytes with okadaic acid, an inhibitor of protein phosphatase 1 and 2A, increased TNF mRNA accumulation without affecting lymphotoxin mRNA levels (Xia et al., 1993). Similarly, cyclosporin A completely inhibited the induction of lymphotoxin but not that of TNF induced by PHA and phorbol ester in peripheral blood lymphocytes (Turner and Feldman 1988). These results differ from Espevik et al. (1987), who showed inhibition of production of both lymphotoxin and TNF by cyclosporin A. Besides cyclosporin, the expression of lymphotoxin has also been shown to be down-modulated by TGF-β in LAK cells (Abe et al., 1992). Like TNF, the production of lymphotoxin from T cells in response to anti-CD3 or concanavalin A has been shown to be down-modulated by prostaglandin E2, a modulation mediated by inhibition of lymphotoxin transcription (Ferreri et al., 1992).

Wong and Goeddel (1986) showed that several viruses can induce lymphotoxin mRNA from peripheral blood leukocytes. Lymphotoxin is secreted by human B cell lines infected with Epstein-Barr virus (EBV; Bersani et al., 1987). In a variety of EBV-transformed B cell lines, PMA causes an increase in lymphotoxin mRNA and protein secretion (Sung et al., 1989). All these studies indicate a direct role for viral activation in production of lymphotoxin from B cell lymphomas. Most of the HTLV-1-transfected T cell lines express lymphotoxin (Tschachler et al., 1989; Paul et al., 1990), and this is mediated through the activation of the NF-kB binding site present in the promoter region of the lymphotoxin gene (Paul et al., 1990). Besides HTLV-1, it has been shown that the HIV-tat gene induces the mRNA and the protein for lymphotoxin in transfected B lymphoblastoid cell lines (Sastry et al., 1990). HIV-tat protein was found to induce the expression of lymphotoxin (Buonaguro et al., 1992), and this required NF-kB and Sp1 binding sites in the lymphotoxin promoter. Interestingly, a stem-loop structure in the lymphotoxin mRNA, which resembles the HIV-1 long terminal repeat (TAR), was also found to be essential for lymphotoxin activation by HIV-tat (Buonaguro et al., 1994), thus showing that similar promoter

regulatory elements are needed for HIV-tat-mediated transactivation of both lymphotoxin and HIV-1 gene expression.

III. CHARACTERIZATION OF LYMPHOTOXIN PROTEIN

Lymphotoxin was originally isolated from a human B cell line as a glycoprotein that had a molecular mass of 60-70 kd under native conditions and 20 kDa and 25 kDa under denaturing conditions (Aggarwal et al., 1983, 1984b, 1985a). It was found that the 25 kDa form of lymphotoxin is a monomeric glycoprotein containing 171 amino acid residues and that it aggregates under native conditions to produce the higher molecular weight trimer (Aggarwal et al., 1985a). The amino acid sequence of the protein revealed that the 20 kDa form is a truncated form of the 25 kDa form, with 23 amino acid residues missing from the amino terminal. Interestingly, this deletion had no affect on its biological activity (Aggarwal et al., 1985a). Both amino acid sequence and lectin-binding results revealed that lymphotoxin is a glycoprotein. Further studies in which carbohydrates were enzymaticallly removed, have demonstrated that lymphotoxin has both N- and O-linked sugars which provides superior solubility but has minimum effect on its biological activity (Haas et al., 1985; Hains and Aggarwal, 1989). The recombinant *Escherichia coli*-derived 25 kDa form of human lymphotoxin has an apparent molecular mass of 18 kDa, a difference due to lack of carbohydrates (Gray et al., 1984; Haas et al., 1985; Seow et al., 1989; Schoenfeld et al., 1991). The delineation of the structure of N-linked sugar chain on recombinant lymphotoxin derived from mammalian cells showed that the sugar moiety has a lectin-like character and plays an important role in the lymphotoxin survival in plasma (Fukushima et al., 1993). The microheterogeneity observed in natural human lymphotoxin has been shown to be due to variable glycosylation at Thr 7, proteolytic processing, and allelic variations (Thr vs. Asn at position 26; Voigt et al., 1992; Matsuyama et al., 1992). Besides being expressed in bacteria and mammalian cells, recombinant lymphotoxin has also been produced in the baculovirus system (Yamashita et al., 1990; Crowe et al., 1994a).

The amino acid sequence predicted from the cDNA indicates that human lymphotoxin has a 34 amino acid signal peptide and a 171 amino acid mature protein with an N-linked glycosylation site at residue 62 and no cysteine residue (Gray et al., 1984; Aggarwal et al., 1985a). This sequence is in agreement with that predicted by several other groups except for a change of asparagine instead of threonine at residue 26 (Kobayashi et al., 1986;

Kato et al., 1989; Matsuyama et al., 1992). This difference may represent a genetic polymorphism that has no affect on lymphotoxin's antigenic or biological characteristics. Although the cDNA for murine, bovine, and rabbit lymphotoxin has also been cloned, these recombinant proteins have not yet been isolated (Goeddel et al., 1986; Li et al., 1987).

The deletion in the amino-terminal of the lymphotoxin molecule was found to have no affect on its biological activity but the deletion of carboxyl terminal 16 (Gray et al., 1984) or 10 (Kobayashi et al., 1986) amino acid residues abrogated lymphotoxin function. This is consistent with the observation that the C-terminal region, which is hydrophobic and less susceptible to proteolytic cleavage, is highly conserved in various species. The structure of lymphotoxin has been resolved by X-ray crystallography at 1.9A° resolution, confirming the earlier reports that lymphotoxin is a trimeric molecule and further showing that each monomer is composed primarily of β-strands (Eck and Sprang 1989). Two sheets formed by eight anti-parallel β strands are arranged in a sandwich structure described as a β-jelly roll.

IV. CHARACTERIZATION OF LYMPHOTOXIN GENE

The amino acid sequence of the protein led to the chemical synthesis of the gene for human lymphotoxin. Using human probes, the cDNA for lymphotoxin from rabbits, mice, and cows have been cloned. Lymphotoxin is encoded by a single gene in the human (Nedwin et al., 1985b; Goeddel et al., 1986; Nedospasov et al., 1986a), mouse (Nedospasov et al., 1986b), cow (Goeddel et al., 1986), and rabbit (Nedospasov et al., 1988) genomes. The lymphotoxin gene is approximately 3 kbp in size and is split by three introns (Nedwin et al., 1985b; Gardner et al., 1987). Like other cytokines, lymphotoxin has an AU-rich motif in the 3' untranslated region that is involved in determining mRNA stability (Caput et al., 1986). The cDNA showed that unlike human lymphotoxin, murine lymphotoxin has a cysteine residue at position 84 that is also conserved in human and murine TNF (Li et al., 1987). Lymphotoxin is the only other cytokine besides TNF whose gene has been mapped within the MHC region (Nedwin et al., 1985b; Spies et al., 1986; Muller et al., 1987; Nedospasov et al., 1985). These genes are tandemly arranged within a 7 kb region in the MHC of all mammalian species studied to date (Nedospasov et al., 1986b).

Several studies have shown high homology in the 5' region of the lymphotoxin gene of the human, rabbit, and mouse especially in the 300

bases upstream of the putative transcription initiation sites (Shakhov et al., 1990; Fashena et al., 1990; Paul et al., 1990). Two functional cap sites within 15 bases of each other have been reported in the murine lymphotoxin gene (Fashena et al., 1990). The predominantly used CAGG cap site has been designated +1 and the location of other regulatory sequences are specified in relation to it. Human, mouse, and rabbit lymphotoxin genes have a TATA box. Within 110 bp upstream of the TATA box is a consensus sequence recognized by the SP1 transcription factor. A near consensus sequence for the AP-2 transcription factor is also present. Several sequences similar to the NF-kB binding site are present in the lymphotoxin gene and have been shown to form complexes when incubated with nuclear extracts from PHA/PMA-activated Jurkat, C81-66-45, and MT-2 cells, all known to contain active NF-kB (Paul et al., 1990). An additional kB sequence is located 3' to the lymphotoxin gene in all species studied. This sequence binds weakly to the NF-kB protein (Collart et al., 1990), but its role in regulation of the gene has not been studied. Interestingly, lymphotoxin itself can activate NF-kB (Chaturvedi et al., 1994), thus suggesting that lymphotoxin can regulate itself.

Additional putative regulatory sequences upstream of the murine lymphotoxin gene have been identified after comparison of the 5' flanking regions of other cytokines and cytokine receptor genes. Similarities have been found among the 5' sequences of the lymphotoxin gene and others that are expressed by TH1 cell subsets. These sequences may reflect common sites for DNA-binding factors and possibily of coordinated regulation. A region of homology in which 22 out of 25 bp are the same appear in the lymphotoxin gene and IL-2 receptor gene upstream sequences. A short lymphotoxin sequence is present in the 5' flanking region of IL-2 which is 9 bp long (Turetskaya et al., 1992). A 7-bp sequence is present in the lymphotoxin, IFN-γ, IL-3, GM-CSF, and IL-2 receptor genes (Gardner et al., 1987). A 178-bp segment (-762 to -584) that is 93% homologous to the mouse alu-like repeat, is present in the lymphotoxin gene. One perfect and two nearly perfect matches to the heat-shock consensus sequences are also noted in 5′ flanking region of the gene (Fashena et al., 1990).

Ruddle and coworkers (Fashena et al., 1990) carried out a 5′ deletion mutation analysis of the murine lymphotoxin gene promoter using chloramphenicol acetyl transferase reporter (CAT) system. They found that a fragment occupying -293 to +77 exhibits promoter activity in transfected murine fibroblasts, the murine lymphomas EL-4 and YAC-1, and the human T cell lymphomas MT-2 and H9. This region of the gene

includes the TATA box, cap site, an inverted Sp1 binding site, a putative AP-2 site, and a 5′-kB-like consensus sequence. But fragments containing further 5′ sequences (-622 to +77, -1186 to +77, and -1186 to +77) exhibit no promoter activity in transfected murine fibroblasts and human and murine T cells. This finding was indicative of the presence of a possible upstream negative regulatory element. This negative regulation is not overcome by cellular activation but is overcome in MT-2 and HTLV-I infected cell lines that produce a high level of constitutive lymphotoxin (Paul et al., 1990). These studies indicate that production of lymphotoxin may occur because of derepression rather than activation.

The previously described deletion mutants were differentially regulated in lymphotoxin-producing murine pre-B cells. The promoter fragment (-293 to +77) did not function in transfected PD and PD31 cells. The larger -662 to +77 and -1186 to +77 fragments exhibit significant promoter activity. This suggests that these fragments contain a sequence that is recognized as an enhancer element in the pre-B, Abelson-infected cells. Removal of a 394-bp region (-662 to -269) abolished the enhancer activity. An upstream enhancing sequence was localized to an AT-rich region between -612 and -580 to which a high mobility group-I-like protein binds (Fashena et al., 1992). The functional promoter fragment of the lymphotoxin gene does not drive CAT expression in transfected mature murine B cells, indicating that either the factors required for lymphotoxin gene promotion are absent or that inhibitory proteins are present in these cells (Turetskaya et al., 1992). Mutational analysis of the 5' NF kB binding site in lymphotoxin transcription indicated that this upstream NF kB binding site is essential for lymphotoxin promoter activity in H9 and MT-2 T cell lines (Paul et al., 1990). The promoter region of the gene and regulation of expression still need to be studied in detail.

Even though there is a single lymphotoxin gene, two major transcripts have been noted in murine CTLL-2 cells activated by IL-2 (Weil et al., 1990). The structure, localization, and kinetics of accumulation of these transcripts revealed that RNA processing is a limiting step for lymphotoxin and that the second lymphotoxin mRNA, coding for a distinct protein, is generated by transport to the cytoplasm of a partially spliced transcript (Weil et al., 1990).

V. POLYMORPHISM IN THE LYMPHOTOXIN GENE

When NcoI digested DNA was hybridized either to lymphotoxin or TNF probes, polymorphic fragments of either 5.5 kb or 10.5 kb were produced

(Webb and Chaplin 1990; Udalova et al., 1993). It was found that the substitution of a G for an A in intron 1 of lymphotoxin produced a new NcoI site (Abraham et al., 1991). Another substitution of an A for C in the 5.5 kb allele resulted in an asparagine instead of threonine at position 26 in mature lymphotoxin (Messer et al., 1991). The 3′ region of lymphotoxin gene has also been found to be polymorphic when EcoRI was used for DNA digestion (Partanen and Koskimies 1988). This restriction fragment length polymorphism (RFLP) was seen at a low frequency. The existence of four microsatellites associated with the lymphotoxin gene has also been noted (Nedospasov et al., 1991). One of these, named TNFc, and consisting of two alleles, one with nine TC repeats and the other with 10, is located in intron 1 of the lymphotoxin gene 3′ from the Nco I site. Two other microsatellites, TNFa and TNFb, are located 3.5 kb upstream of lymphotoxin gene. These have seven and 13 alleles, respectively (Jongeneel et al., 1991). The role and significance of these sequences is not known.

VI. LYMPHOTOXIN-RELATED MOLECULES

As indicated previously, the antibodies against the highly purified lymphotoxin led to the identification of a second cytotoxic factor that was called TNF (Aggarwal et al., 1985b; Stone-Wolff et al., 1984). The structural determination of lymphotoxin and TNF revealed that these two cytokines are 28% identical and as much as 51% homologous at amino acid sequence levels (Aggarwal et al., 1985a; Pennica et al., 1984; Gray et al., 1984). The homology was found to be even higher at the nucleotide level and the genes for both were found to be of similar size with three introns each and were closely-linked in the MHC-region on chromosome 6. This homology of lymphotoxin to TNF was not just restricted to their structure but also found in their function. Both lymphotoxin and TNF were found to display similar antitumor properties *in vitro* and *in vivo* (Pennica et al., 1984; Gray et al., 1984), and were shown to share a common cell surface receptor (Aggarwal et al., 1985c).

There is, however, an important difference between the synthesis of TNF and lymphotoxin. The former is synthesized as a type II transmembrane protein which then undergoes proteolytic cleavage causing the release of secreted TNF (Kriegler et al., 1988). In comparison, lymphotoxin is synthesized directly as a secreted protein. Recently, a cell-surface form of lymphotoxin has been discovered which results from its binding to a non-receptor transmembrane

glycopeptide of 33kDa, thus forming a heteromeric complex (Browning et al., 1991; Androlewicz et al., 1992).

Interestingly, the cloning of the cDNA of this lymphotoxin binding peptide revealed that its predicted amino acid sequence has homology to that of lymphotoxin and TNF. Although no functional homology has been noted between this lymphotoxin-binding peptide and lymphotoxin to date, based on structural homology it was named as lymphotoxin-β (Browning et al., 1993). This cDNA encodes a 240-244 amino acid sequence (molecular mass of 25-26 kDa) typical of type II transmembrane proteins. The lymphotoxin-β gene is approximately 2 kb long and very closely linked to lymphotoxin and TNF genes in the MHC region. It is situated within 2 kb of the TNF gene, has four exons, and its arrangement is very similar to that of lymphotoxin and TNF genes except for its opposite orientation. It has been shown that one molecule of lymphotoxin associates with two molecules of LTβ to form a heterotrimeric complex (Browning et al., 1995; Figure 1). Whether lymphotoxin-β is a cytokine or a cytokine receptor is not clear; the only function assigned to this molecule to date is to bind secreted lymphotoxin in certain types of T cells.

The other members of the lymphotoxin/TNF ligand family include ligands for CD40, CD30, CD27, 4-1BB, OX 40, and Fas (Armitage et al., 1992; Smith et al., 1993; Goodwin et al., 1993; Suda et al., 1993, Alderson et al., 1994; Baum et al., 1994; Figure 2). All these proteins are type II transmembrane glycoproteins with homology to each other in their C-terminal extracellular domains, ranging from 12-29% with particular conservation within the β-strand regions, including the residues in lymphotoxin and TNF that are involved in intersubunit contacts. This analysis strongly suggests that all these related ligands share the same β sandwich structure and may form trimers like lymphotoxin and TNF.

VII. LYMPHOTOXIN RECEPTORS

Soon after highly purified natural and recombinant lymphotoxin became available, radiolabeled cytokine showed that lymphotoxin binds cells with high affinity (Haas et al., 1985; Stauber and Aggarwal, 1989; Hirano et al., 1989). A single class of receptors with an affinity of 0.1-1 nM and a density of 1,000-5,000 binding sites per cell was identified. Competition studies showed that the receptors for lymphotoxin are the same as those for TNF (Aggarwal et al., 1985c), thus explaining the similarities

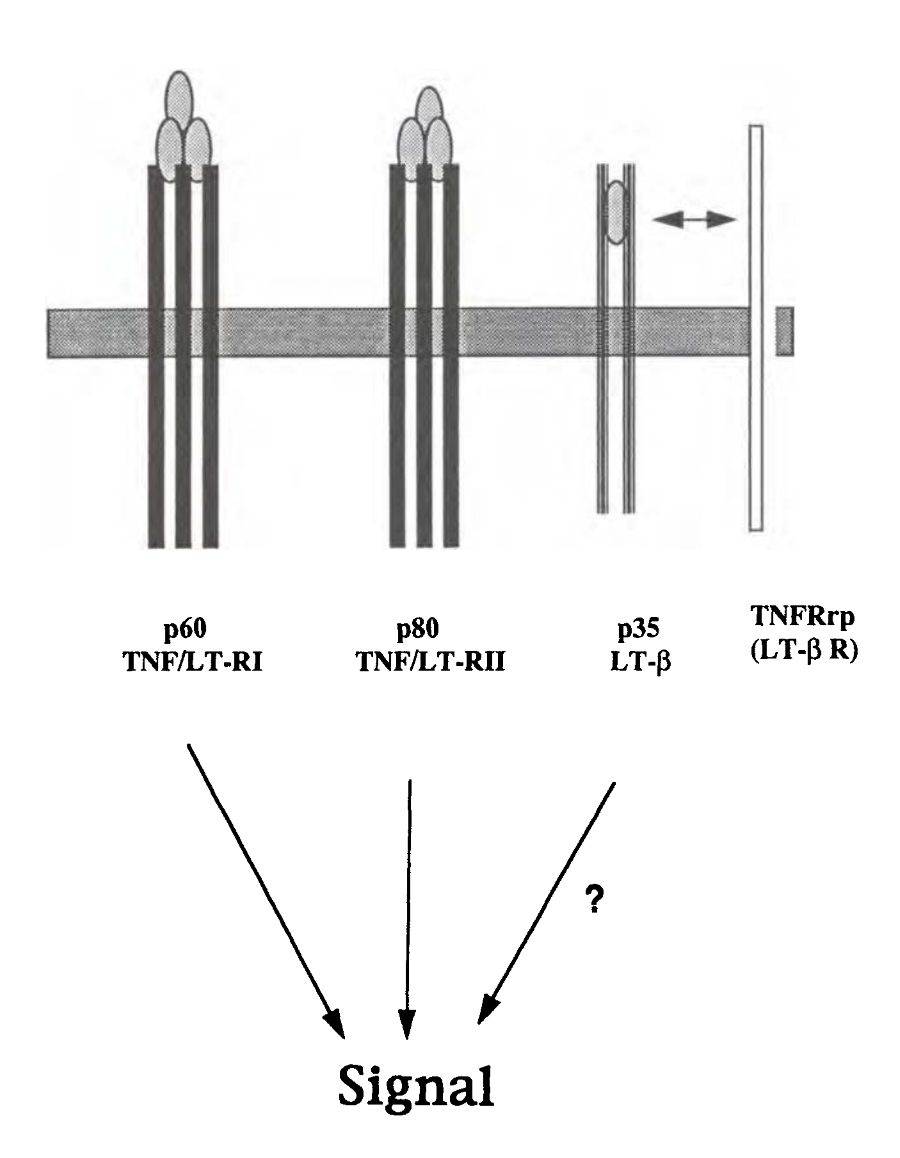


Figure 1. Interaction of lymphotoxin with various transmembrane proteins.

in the activities of the two cytokines (for references see Aggarwal and Vilcek, 1992). Almost all cell types have been shown to display receptors for lymphotoxin/TNF except human red blood cells (Shalaby et al., 1987). These receptors are up-regulated by a wide variety of agents, including interferons, interleukins, colony-simulating factors, protein kinase C inhibitors, intracellular cAMP elevating agents, and lectins. (Aggarwal et al., 1985c; Ruggiero et al., 1986; Scheurich et al., 1987; Tsujimoto et al., 1986a, 1986b; Tsujimoto and Vilcek, 1986; Aggarwal and Eessalu, 1987a; Aggarwal et al., 1986; Owen-Schaub et al., 1989; Pang et al., 1989; Zhang et al., 1994). In contrast, lymphotoxin/TNF receptors are down-regulated by IL-1, phorbol ester, okadaic acid, TNF, thiol reagents, and lipopolysaccharide (Aggarwal et al., 1987a, 1987b; Holtmann and Wallach, 1987; Tsujimoto and Vilcek, 1987; Ding et al., 1989; Higuchi and Aggarwal, 1993, 1994; Zhang and Aggarwal, 1994). A cell-type dependent regulation of lymphotoxin/TNF receptors has also been noted. For instance, while in most cells interferons up-regulate these receptors, in HepG2 cells, IFN-γ down-modulates the receptors (Aggarwal and Pandita, 1994). Since only a fraction of the receptors are needed for the transduction of biological response (Chan and Aggarwal, 1994), the modulation of receptors does not always lead to the modulation of biological response (Aggarwal and Eessalu, 1987a).

Two different receptors for lymphotoxin/TNF with a molecular mass of approximately 60 kDa (referred to as p60 or TNFR1) and 80 kDa (referred to as p80 or TNFR2) have been identified (Figure 1). Lymphotoxin and TNF both bind to these receptors independently with high affinity (Loetscher et al., 1990; Schall et al., 1990; Smith et al., 1990; Nophar et al., 1990; Heller et al., 1990; Kohno et al., 1990; Himmler et al., 1990). In general, most cells express both receptor types but in variable proportion: p60 is primarily expressed by epithelial cells, whereas p80 is expressed mainly by myeloid cells (Hohmann et al., 1989; Brockhaus et al., 1990). The amino acid sequence of the p60 receptor predicted from the cloned cDNA consists of 426 amino acids with a single membrane span, an extracellular domain (ECD) of 182 amino acids, and an intracellular domain of 221 amino acids. The p80 receptor is 439 amino acid residues long with a single membrane spanning domain, an ECD of 235 amino acids and an intracellular domain of 174 amino acids. Both N- and O-linked glycosylation occur on the ECD of both the receptors. The mouse p60 and p80 cDNAs have also been isolated (Lewis et al., 1991; Goodwin et al., 1991) and found to be 64% and 68% identical to their respective human counterparts. The p60

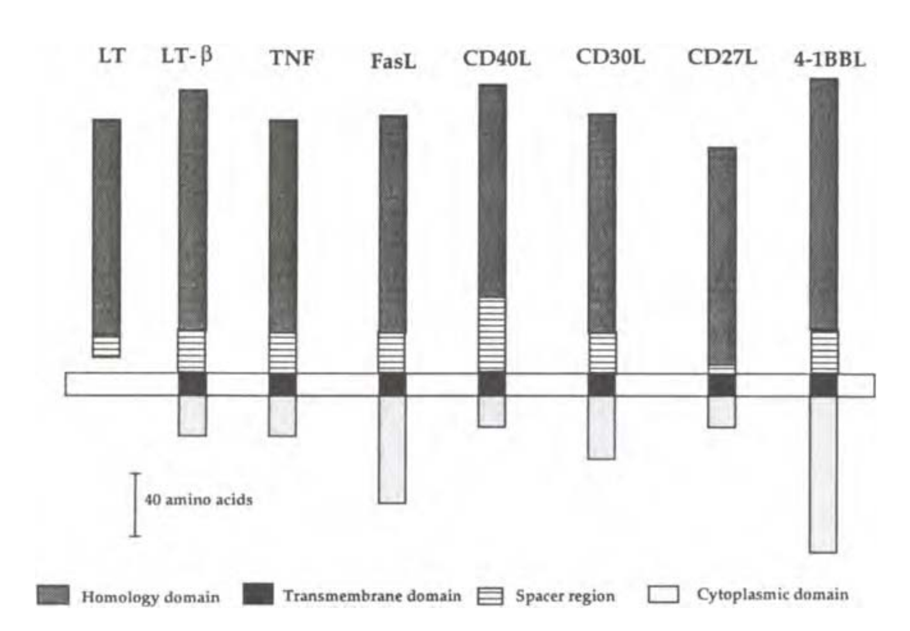


Figure 2. Lymphotoxin and its various family members.

receptor is most conserved between human and murine in the ECD (70% identity), while p80 is most conserved in the intracellular domain (73% identity). There is a complete absence of homology between the intracellular domains of the two receptors, suggesting that they utilize distinct signaling pathways (Lewis et al., 1991).

To identify the structural features important for signaling, deletion mutagenesis of the intracellular regions of both receptors has been carried out (Tartaglia et al., 1993, Brakebusch et al., 1992; Rothe et al., 1994). These studies revealed that about 80 amino acids near the C terminus are responsible for signaling cytotoxicity through the p60 receptor. This domain has a weak homology with the intracellular domain of the fas antigen, and many of the amino acids conserved in the intracellular domain of the p60 and the fas antigen are critical for the cell death signal. Thus, this region has been named the "death domain." Mutation in this domain of p60 disrupts its ability to transduce antiviral activity and NO synthase induction, whereas large deletions in the

membrane-proximal half of the intracellular domain do not abrogate signaling of cytotoxicity or antiviral activity but rather abolish the induction of NO synthase. Similar analysis with the p80 receptor revealed that a deletion of the carboxyl terminal 16 amino acids of the cytoplasmic domain did not affect signaling, but deletion of 37 amino acid residues at that end abolished signaling.

In addition, two proteins that constitutively associate with the p80 receptor have been identified, but their role in signaling has not been established (Rothe et al., 1994). We have reported a physical and functional association of two distinct protein kinases with the cytoplasmic domain of the p60 and p80 receptor (Darnay et al., 1994a, 1994b).

Lymphotoxin is the one of the few cytokines that has been co-crystallized with its receptor and whose structure as a complex has been solved (Banner et al., 1993). The structure determined at 2.85 A0 resolution showed that three molecules of the p60 receptor bound symmetrically to one lymphotoxin trimer. The receptor binds in the groove between two adjacent lymphotoxin subunits. The structure of this complex reflects the activated state of the receptor at the cell surface and provides a model for lymphotoxin signal transduction.

VIII. LYMPHOTOXIN/TNF RECEPTOR-RELATED PROTEINS

Besides p60 and p80, several other members of this family that have been identified (Figure 3) including receptors for nerve growth factor (Johnson et al., 1986), CD40 (Stamenkovic et al., 1989), CD30 (Dürkop et al., 1992), CD27 (Camerini et al., 1991), 4-1BB (Kwon and Weissman, 1989), fas (Itoh et al., 1991), and OX40 (Mallett et al., 1990). In addition, a number of members of the poxvirus family have been shown to encode for a secreted form of the lymphotoxin/TNF receptor with strong homology to the extracellular domain of these receptors (Upton et al., 1991; Smith et al., 1990, 1991). Recently, a cowpox virus containing two copies of a gene encoding a soluble secreted form of p80 has been reported (Hu et al., 1994). These receptors are type I membrane glycoproteins and are characterized by the presence of variable number of repeated domains of about 40 amino acids. Their distinguishing feature is 3-5 cysteine-rich domains with a characteristic spacing pattern. Additional amino acid homologies can be recognized between domains in the same or different receptors, so that overall homologies between family members within

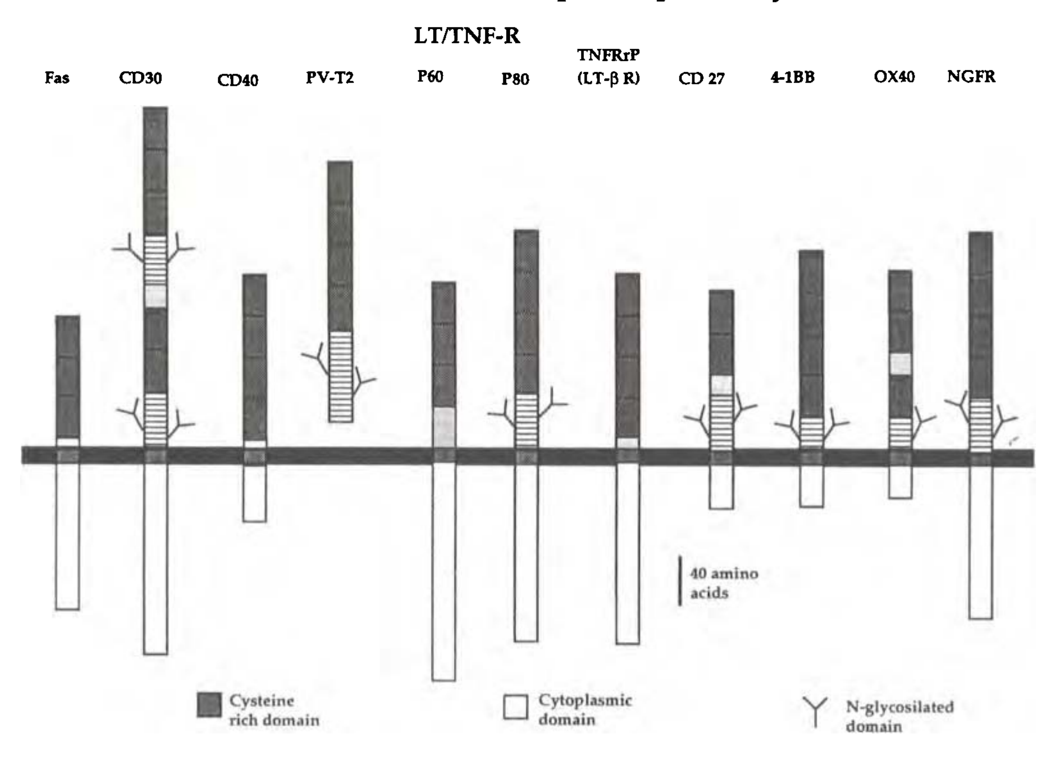


Figure 3. Lymphotoxin/TNF receptors and their family members.

these domains are in the range of 25%. These cysteine-rich domains constitute the ligand-binding portion of the receptor. C-terminal to the cysteine-rich domains are stretches of amino acids of variable length that are rich in proline. Serine and threonine residues are potential sites for additional O-linked glycosylation. CD30 is unusual in that cysteine-rich and O-linked sugar rich regions are partially duplicated.

The cytoplasmic domains of these receptors are of variable length and show no homology to other proteins of known junction. A highly limited sequence homology has been recognized between p60, fas, and CD40. The death domain responsible for triggering cell death, has been defined in the fas cytoplasmic domain, which shows homology to p60 (Itoh et al., 1993; Tartaglia et al., 1993). The cytoplasmic domains of 4-1BB and CD27 also share a sequence homology (Gravestein et al., 1993). The sequence homology observed between the members of this receptor family suggests that these genes evolved from a common ancestor by duplication and divergence.

In addition to the previously mentioned family members, another receptor for heterotrimeric complex of lymphotoxin and lymphotoxin-β (referred as lymphotoxin-β R) had been identified on T cells (Figure 1) that has a CXXCXXC homology to p60 and a homology in the loop created by the disulfide bonds in domains 1 and 2 (Crowe et al., 1994b). The latter is the major contact domain for lymphotoxin (Banner et al., 1993). In contrast, domains 3 and 4 of this novel receptor resemble those of p80 in the positioning of the cysteine residues and by the presence of a shortened loop 1 (5 vs. 15 residues). An additional similarity between LTβ R and p80 is the membrane-proximal proline-rich region in the extracellular domain, which is thought to form a stalk extending to the receptor from the cell surface. The cytoplasmic region of the LTβ R has some similarity to the death domain but very little sequence similarity with other members of this receptor superfamily, which suggests that the mechanism used to signal responses may diverge from the lymphotoxin/TNF receptor.

IX. BIOLOGICAL ROLE OF LYMPHOTOXIN

Because lymphotoxin mediates its signal through the same receptor as TNF, it is generally assumed that both cytokines exhibit similar biological activities. Therefore, most of the studies carried out during last decade have been with TNF. However, there are some studies which have been carried out with either lymphotoxin in the context of TNF or with lymphotoxin alone (Figure 4).

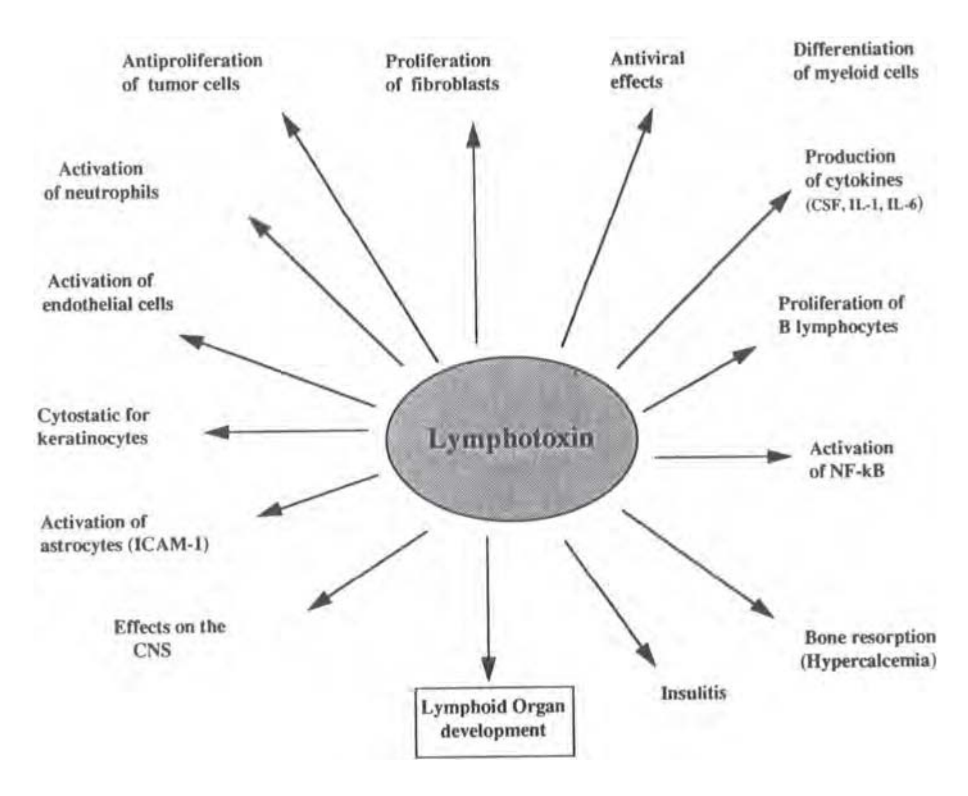


Figure 4. Biological properties of lymphotoxin as determined by *in vitro* experiments. The role of lymphotoxin in insulitis was determined by transgene studies. The activity indicated in a box was determined from lymphotoxin gene knock-out animals. CNS is the central nervous system.

A. Lymphotoxin Exhibits Antiproliferative Activity Against Tumor Cells

Like TNF, recombinant human lymphotoxin inhibits the growth of several different tumor cell lines. In addition to murine fibrosarcoma L929 cells, it inhibits human lung carcinoma (A549), SV-40 transformed human lung fibroblast (WI-38 VA 13), and murine melanoma cells (B16; Aggarwal et al., 1984a; Lee et al., 1984). On a number of different tumor cells lymphotoxin is more potent cytotoxic agent than TNF (Kramer et al., 1986; Beran et al., 1987; Browning and Ribolini, 1989). The antiproliferative effects of lymphotoxin were found to be synergistic with interferon (IFN)-γ (Williams and Bellanti, 1984; Stone-Wolff et al., 1984; Lee et al., 1984; Browning and Ribolini, 1989; Kawatsu et al, 1990a). Since IFN-γ induces receptors for lymphotoxin/TNF (Aggarwal et al., 1985c), it is generally assumed by many investigators that this is responsible for their synergistic anticellular effects, but we have shown that this is not the case (Aggarwal and Eessalu, 1987a).

B. Lymphotoxin as B Cell Growth Factor

Several reports suggest that lymphotoxin is a growth factor for normal B cells, B-lymphoblastoid cells, and B cell lymphomas. One of the first reports on lymphotoxin as a growth factor for human B lymphocytes was given by Kehrl et al. (1987). In certain human B-lymphoblastoid cells, lymphotoxin serves as an autocrine growth factor (Seregina et al., 1989) and lymphotoxin was found to be as potent as TNF in stimulating the growth of B cell chronic lymphocytic leukemia (Moberts et al., 1989). In the presence of other stimuli, lymphotoxin induces activation, proliferation, and differentiation of B lymphocytes (Zola and Nikoloutsopoulos, 1989).

We have recently shown that lymphotoxin is a potent autocrine growth factor for EBV-infected B cells derived from patients with acute lymphoblastic leukemia, myelodysplastic syndrome, and acute myelogenous leukemia (Estrov et al., 1993). Interestingly, in contrast to lymphotoxin, these cells did not produce TNF, nor was their growth stimulated by TNF. Instead TNF was found to be a competitive antagonist in inhibiting lymphotoxin-mediated cellular growth.

C. Lymphotoxin Induces Proliferation and Differentiation of Hematopoietic Cells

It has been shown that lymphotoxin can inhibit colony formation by erythroid and multipotential precursor cells (Murphy et al., 1988). On day 14, TNF was a more potent inhibitor of this activity than lymphotoxin. A low concentration of lymphotoxin has been shown to stimulate the growth of normal CFU-GM, whereas higher concentrations inhibit it (Gullberg and Nilsson, 1989). TNF, in contrast, was found to be only inhibitory in these studies, thus showing qualitative differences between the effects of two cytokines on hematopoietic cells. Both lymphotoxin and TNF inhibited growth of chronic myeloid leukemia cells to the same extent, however.

Lymphotoxin induces the differentiation of human myeloid leukemia cell lines (HL-60 and THP-1) without being cytotoxic (Trinchieri et al., 1986; Hemmi et al., 1987; Shimizu et al., 1989). After lymphotoxin exposure, HL-60 cells express nonspecific esterase, reduce nitro blue tetrazolium, express IgG Fc receptor, and become phagocytic for sheep red blood cells (Hemmi et al., 1987). Similar to the effects of TNF, IFN-γ also potentiated the effects of lymphotoxin (Trinchieri et al., 1986). Takeda et al. (1994) recently showed that lymphotoxin is significantly less potent than TNF in inducing differentiation of human myeloblastic leukemia ML-1 cells. This is in agreement with a report from our laboratory, in which we showed that for reduction of nitro tetrazolium blue (NBT) in ML-1a cells, lymphotoxin is several-fold less potent than TNF (Chaturvedi et al, 1994).

D. Lymphotoxin Activates Neutrophils

Several reports indicate that lymphotoxin, like TNF, can interact with neutrophils and activate them for antibody-dependent cell-mediated cytotoxicity (ADCC) and for superoxide radical production. However, in this system TNF always appears to be more active on a molar basis than lymphotoxin (Shalaby et al., 1985, 1987; Figari et al., 1987; Kapp et al., 1989; Kownatzki et al., 1988). Unlike TNF, however, lymphotoxin lacks chemotactic activity for neutrophils (Ferrante et al., 1988; Mrowietz et al., 1989). Exposure of neutrophils to lymphotoxin leads to the secretion of granule proteins, that is, lactoferrin and myeloperoxidase (MPO; Richter, 1990). Interestingly, the lymphotoxin-induced secretion of MPO was found to be inhibited by pertussis toxin, which blocks

G-protein, whereas lactoferrin secretion was abrogated only by trifluoroperzine, which inhibits both protein kinase C and calmodulin.

E. Effect of Lymphotoxin on T Lymphocytes

T lymphocytes are known to express lymphotoxin which acts in an autocrine fashion to modulate T cell responses during antigenic stimulation. Treatment of a human CD4+ T cell hybridoma with TNF causes the induction of MHC class I gene expression, whereas lymphotoxin has no effect on these cells (Andrews et al., 1990). This difference was assigned to differences in the binding property of TNF and lymphotoxin to these cells. In addition, human lymphotoxin was found to induce the differentiation of IL-2-dependent lymphokine-dependent killer cells (LAK) by increasing the CD4+ and decreasing CD8+ cell population (Abe et al., 1993).

F. Lymphotoxin Modulates Endothelial Cell Function

There are several reports indicating that lymphotoxin can interact with endothelial cells and modulate their functions. It promotes the adherence of endothelial cells to neutrophils. Lymphotoxin interacts with endothelial cells quite differently from the way TNF interacts (Broudy et al., 1987; Locksley et al., 1987; Desch et al., 1990). For instance, severalfold higher concentrations of lymphotoxin are needed for induction of IL-1 and TNF induces hematopoietic growth factor production whereas lymphotoxin was found to be completely ineffective in this regard. Pober et al. (1987), however, found the effects of lymphotoxin on cytokine production and induction of adhesion molecules quite comparable with TNF. Although the basis for differences in results is not clear, the source of lymphotoxin, whether natural or recombinant, glycosylated or nonglycosylated, or degree of purity are some of the factors that may play important roles. Lymphotoxin was also found to increase the permeability of human vascular endothelial cells at comparable levels with TNF (Shinjo et al., 1989).

G. Lymphotoxin Modulates Fibroblast Cell Function

Like TNF, lymphotoxin also causes proliferation of normal human fibroblast cells (Lee et al., 1984; Hofsli et al., 1987). Lymphotoxin activates fibroblasts to produce GM-CSF, M-CSF, and IL-6. However,

it is significantly less potent in this system than TNF (Zucali et al., 1988; Akashi et al., 1989, 1990).

H. Lymphotoxin is Cytostatic for Keratinocyte

It is intriguing that while lymphotoxin stimulates the growth of normal fibroblasts, it inhibits the proliferation of normal human keratinocytes *in vitro* in a dose-dependent manner and in this action synergizes with IFN-γ (Symington, 1989). Lymphotoxin and TNF in this system were found to be quite comparable (Pillai et al., 1989). These observations have relevance to graft-versus-host disease (GVHD), which is characterized by a sequence of epithelial atrophy and fibrosis.

I. Role of Lymphotoxin in Bone Resorption

Human myeloma cells in culture can produce lymphotoxin (Garrett et al., 1987), which may play an important role in bone resorption. Remodeling of the bone occurs in discrete and localized units throughout the skeleton via local factors that are regulated by osteoclast and osteoblast activity. Immune cells appear to be very suitably located as sources for mediators of these activities. Bertolini et al. (1986), using ^{45}Ca, observed that lymphotoxin stimulates osteoclastic bone resorption in organ cultures. Lymphotoxin was found to cause hypercalcemia (Garret et al., 1987) and to act as an osteoclast-activating factor in patients with adult T cell leukemia. Besides, lymphotoxin levels have been shown to be frequently elevated in the serum of adult T cell leukemia patients with hypercalcemia (Ishibashi et al., 1991). Lymphotoxin production by HTLV-1 infected cell lines implicated a role for lymphotoxin in HTLV-I-associated clinical manifestations such as adult T cell leukemia-associated hypercalcemia and tropical spastic paraparesis-associated demyelination. Pfeilschifter et al. (1989), using a long-term human marrow mononuclear cell culture system, found that lymphotoxin increases both the proliferation of marrow mononuclear cells and the differentiation of committed progenitors in this system.

Like TNF, lymphotoxin at a concentration of 10^{-7}-10^{-9}M causes a decrease in collagenase-digestible protein synthesis in fetal rat calvaria (Bertolini et al., 1986). Smith et al. (1987) found that lymphotoxin inhibits alkaline phosphatase activity and collagen synthesis in rat osteosarcoma cells but stimulates cell proliferation, indicating that lymphotoxin also stimulates cells with an osteoblast phenotype. The findings

of Yan and Huang (1991) suggest a possible role for lymphotoxin in human middle ear cholesteatoma.

J. Lymphotoxin as an Antiviral Agent

Paradoxically, viruses have been shown to induce LT expression and in turn lymphotoxin displays antiviral activity against both DNA (adenovirus and herpes simplex virus type II) and RNA (encephalocarditis virus and vesicular stomatitis virus) viruses (Wong and Goeddel, 1986). It also synergizes with interferons in the induction of resistance to infection by these viruses in diverse cell types. These effects of lymphotoxin were not due to induction of IFN synthesis. In addition, lymphotoxin has been shown to selectively kill the virally infected cells. No quantitative differences between lymphotoxin and TNF have been noted in these effects.

K. Lymphotoxin Exhibits Lipolytic Effects on Adipocytes

It has been shown that TNF has lipolytic effects on adipose cells, and that this is responsible for the cachectic activity of this cytokine (Beutler et al., 1985). Like TNF, lymphotoxin was also found to induce lipolysis in fat cells (Patton et al., 1986), however, direct evidence of a role for lymphotoxin in cachexia has not been reported.

L. Effects of Lymphotoxin *In Vivo*

The usefulness of lymphotoxin for growth inhibition of human and murine solid tumors and in experimental metastasis in mice has been demonstrated (Funahashi et al., 1993). A highly purified bacteria-derived recombinant human lymphotoxin administered to mice bearing methylcholanthrene A (Meth A)-induced sarcomas was found to cause hemorrhagic necrosis of the tumors (Gray et al., 1984). This is an activity similar to that described for recombinant human TNF (Pennica et al., 1984). Another recent study confirmed that systemic administration of lymphotoxin to BALB/c mice exhibits a significant anti-tumor activity against Meth A sarcoma (Funahashi et al., 1993). Interestingly, during these studies a five times higher dose of TNF and nonglycosylated lymphotoxin was needed to attain the same degree of effectiveness as glycosylated lymphotoxin. This difference may in part be due to the threefold longer serum half-life of glycosylated lymphotoxin compared

with nonglycosylated lymphotoxin and 22-fold longer half-life compared to TNF, which is naturally nonglycosylated. These results are consistent with that reported by Kawatsu et al. (1990b) in rats. Besides antitumor activity, these studies clearly demonstrate the importance of the sugar moiety in the mechanism of action of this cytokine. Lymphotoxin also was found to increase the survival of animals bearing L-1210 leukemia (Lee et al., 1984). As compared with control animals, spleen cells from mice bearing progressively growing syngeneic sarcomas showed expression of TNF mRNA but not lymphotoxin mRNA (Lipoldova et al., 1993), suggesting selective impairment of T cell function.

Similarly to the *in vitro* results, administration of lymphotoxin to mice induces production of GM-CSF, M-CSF, and IL-1 (Kaushansky et al., 1988). Lymphotoxin has been shown to induce reactive nitrogen intermediates in mice (Rockett et al., 1992), it is elevated in the serum of malaria patients and thus is thought to be responsible for the increase in plasma IL-6 in these patients (Clark et al., 1992).

Recombinant human lymphotoxin given intravenously to Lewis rats induces peripheral neutrophilia and lymphopenia in a dose-dependent manner (Ulich et al., 1987). This reaction is accompanied by depletion of bone marrow neutrophils. Changes in circulating leukocytes are not due to hemodynamic changes because lymphotoxin has no effect on systemic blood pressure. Lymphotoxin has also been administered to anesthetized sheep to examine its effects on systemic and pulmonary hemodynamics, lung lymph dynamics, and eicosanoid release. Interestingly, it was found that, in contrast to TNF, lymphotoxin did not increase lung lymph and plasma levels of 6-keto-prostaglandin F1a and, also unlike TNF, did not cause systemic vasodilation and hypotension (Kreil et al., 1989). Again unlike TNF, lymphotoxin had no effect on lymph thromboxane B2 levels. All these results clearly indicate that TNF and lymphotoxin behave differently not only *in vitro* but also *in vivo*.

An acute thymic involution or atrophy mainly observed in patients under physical stress or with bacterial infections or malignancies has been thought to be induced by various mediators. Lymphotoxin in combination with estrogen induced profound thymic involution even in adrenalectomized mice without inducing lymphocytopenia in the peripheral organs (Hirahara et al., 1994). These observations suggest that lymphotoxin might be an important regulator of the immune system.

X. DISSIMILARITIES IN ACTIVITIES BETWEEN LYMPHOTOXIN AND TNF

Even though very few reports compare the effects of TNF with lymphotoxin or examine the biological role of lymphotoxin alone, the latter differs from TNF both qualitatively and quantitatively. For instance, the role of TNF in cachexia, septic shock, cerebral malaria, and several other pathological conditions is known. Whether lymphotoxin is also involved in any of these situations, is not understood. Similarly, TNF is frequently detected in the synovial fluids and in serum of rheumatoid arthritis patients and believed to play an important role in pathogenesis, however, no lymphotoxin was found in such patients (Saxne et al., 1988). TNF has been shown to inhibit the development of *Trypanosoma brucei brucei in vitro* and *in vivo*; whereas lymphotoxin does not display this activity (Lucas et al., 1994). A close structural analysis of the TNF and lymphotoxin demonstrated that this activity is localized in a region of TNF (Ser99 to Glu115 of murine and Ser100 to Glu116 in humans) that is absent from lymphotoxin (Lucas et al., 1994).

TNF was found to stimulate the growth of tumor cells in hairy cell leukemia but lymphotoxin had no effect (Buck et al., 1990), and several human tumor cell lines have also been shown to respond differently to lymphotoxin and TNF (Browning and Ribolini, 1989). The growth of human acute myelogenous leukemia cells (KBM-5) and colon cancer cells (LoVo) are minimally affected by lymphotoxin but dramatically inhibited by TNF (Beran et al., 1987; Matsubara et al., 1990). Also on human smooth muscle cells, TNF has been shown to induce HLA-DR expression whereas lymphotoxin was ineffective (Stemme et al., 1990).

Lymphotoxin and TNF had opposite effects on the central nervous system: TNF decreased whereas lymphotoxin increased the activity of sympathetic efferent nerves to intercapsular brown adipose tissue (Holt et al., 1989). TNF was found to induce the differentiation of neuroblastoma cells (N103) but lymphotoxin was completely ineffective (Muñoz-Fernandez et al., 1994). This differentiation was found to be mediated through NO. For induction of intercellular adhesion molecule (ICAM-1) expression in astrocytes, lymphotoxin was found to be 10 to 100-fold less potent than TNF (Frohman et al., 1989).

The expression of various cytokines (e.g., M-CSF, G-CSF, GM-CSF, IL-1, and IL- 6) also appears to be regulated differently by lymphotoxin and TNF (Koeffler et al., 1987; Mantovani et al., , 1990; Brach et al., 1990). In macrophages, TNF, but not lymphotoxin, is known to induce

the secretion of M-CSF; instead lymphotoxin blocks the TNF-induced effect by acting as a competitive inhibitor (Oster et al., 1987). TNF has been shown to induce IL-6 in neutrophils whereas lymphotoxin has no effect (Cicco et al., 1990). Differences are also observed in endothelial cells, where TNF was found to be an active inducer of hematopoietic growth factors and IL-1 and to promote neutrophil adhesion; once again lymphotoxin was found to be minimally effective (Locksley et al., 1987; Broudy et al., 1987).

Why activities of lymphotoxin should differ from those of TNF is not clear. In some instances, the differences are due to differences in their binding affinities (Kircheis et al., 1992; Andrews et al., 1990) or to structural differences (Lucas et al., 1994). However, other studies suggest that receptor affinity alone is not sufficient to explain such differences (Chaturvedi et al., 1994). The residues needed to constitute the framework for the trimeric structure of lymphotoxin have been well conserved with TNF (Tavernier et al., 1989) and some of the residues such as tryptophan which are conserved have been shown by site-directed mutagenesis not to be essential for biological activity (Van Ostade et al., 1988). By receptor transfection studies it was found that the p60 form of the lymphotoxin/TNF receptor can distinguish between lymphotoxin and TNF (Schuchmann et al., 1994). The antibodies that bind to the p60 receptor can block TNF binding but not lymphotoxin binding (Espevik et al., 1990), also suggesting that there are distinct domains on the p60 receptor that bind TNF and lymphotoxin. Iwamoto et al. (1994) concluded from their studies that lymphotoxin signaling occurs only through the p60 receptor and not through the p80 receptor.

XI. ROLE OF LYMPHOTOXIN AS LEARNED FROM GENE KNOCK-OUT EXPERIMENTS

To define the role of the lymphotoxin gene, De Togni et al. (1994) generated mice deficient in lymphotoxin by the gene targeting technique. They found that mice with the lymphotoxin -/- genotype showed abnormal development of peripheral lymphoid organs. No popliteal, inguinal, para-aortic, mesenteric, axillary, or cervical lymph nodes could be seen in these mice. Morphological changes were also seen in the spleen of these mice. At the age of six weeks, the number of nucleated spleen cells in these mice was similar in both wild-type and lymphotoxin-deficient mice, but the spleen architecture and B and T lymphocyte content

differed. The spleens of lymphotoxin -/- mice contained slightly fewer T lymphocytes and more B lymphocytes. By histological analysis, the spleens of lymphotoxin-/- mice showed changes in the white pulp with altered organization of the periarteriolar T cell zone and loss of a distinct marginal zone. The peripheral blood of lymphotoxin -/- mice was found to contain nearly fourfold more B cells than did that of normal mice, and the total white blood cell count was almost threefold higher in lymphotoxin-deficient mice.

The mechanism by which lymphotoxin contributes to normal lymphoid organ development is not understood. The requirement of p60 and p80 form of the lymphotoxin/TNF receptors in this action has been ruled out because mice deficient in either of these receptors do not show changes similar to that observed during lymphotoxin deficiency (Pfeffer et al., 1993; Erickson et al., 1994). Whether lymphotoxin-β receptor or some other polypeptideis is responsible for the differences is not completely understood.

Mutant mice deficient in both TNF and lymphotoxin have also been generated by the gene targeting technique (Eugster, 1994). The double mutant mice showed no obvious abnormailty up to four weeks of age and were found to be fully protected from endotoxin shock, just as p60 receptor-deficient mice are (Pfeffer et al., 1993).

XII. EARLY EVENTS IN THE SIGNAL TRANSDUCTION OF LYMPHOTOXIN

Because of the structural similarity and common cell surface receptor, most of the studies on signal transduction have been carried out with TNF. When lymphotoxin was compared with TNF, some very interesting differences were noted. For instance, TNF activates neutral sphingomylinase within 2.5-5 min of exposure to human skin fibroblasts whereas lymphotoxin under these conditions has no effect (Chatterjee, 1994). TNF activation of the nuclear transcriptional factor NF-kB, one of the early events mediated by both TNF and lymphotoxin (Hohmann et al., 1990), occurs within minutes in ML-1a cells, but lymphotoxin activation did not (Chaturvedi et al., 1994). When cells were treated for longer times and with 1,000 to 10,000-fold higher concentration, lymphotoxin does activate NF-kB but the activated complex was found to be short-lived. Why this difference in the action of lymphotoxin and TNF should exist is not clear at present. These differences cannot be explained

based on their affinity to the receptor but it is possible that there is another signaling subunit that distinguishes between TNF and lymphotoxin. In fact, a transmembrane protein p35, also referred to as lymphotoxin-β, has been identified in T cells. It interacts with lymphotoxin but not TNF (Browning et al., 1991). However, the expression of lymphotoxin-β is restricted to only T, B, and NK cells (Ware et al., 1992) and, furthermore, no physical or functional association between lymphotoxin/TNF receptor and lymphotoxin-β has yet been established. Therefore, the signaling apparatus for lymphotoxin and the basis for its differences from TNF are not clear. Like TNF, however, lymphotoxin binds to planar lipid bilayer and forms ion channels in a pH-dependent manner (Baldwin et al., 1995).

XIII. PATHOLOGICAL ROLE OF LYMPHOTOXIN

Lymphotoxin has been shown to play an important role in inflammation and in the development of autoimmune diseases such as insulin-dependent diabetes mellitus and rheumatoid arthritis. It has been shown that transgenic mice expressing lymphotoxin in the pancreas develop insulitis but not diabetes (Picarella et al., 1992; Ruddle et al., 1993). Instances of disease with both higher and lower levels than normal of this cytokine have been noted. It would be useful to understand the basis of these associations and correlations with particular diseases so as to elucidate their pathogenesis. The genes encoding lymphotoxin and TNF are located within the major histocompatibility complex, and the gene polymorphism of lymphotoxin and TNF have been found to be associated with autoimmune disease. Genetic markers have been used to find the correlation between disease and lymphotoxin at the genetic level. In the case of diabetes mellitus with $B15^+$, $DR\text{-}4^+$ haplotypes, the 10.5 kb allele was found in an increased frequency (Pociot et al., 1991). The 8.1 kb haplotype (5.5 kb allele) has been associated with autoimmune diseases like rheumatoid arthritis (Abraham et al., 1991). To find out whether these alleles have any bearing on the level of lymphotoxin, studies have been undertaken. Phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells from individuals who were homozygous for the 5.5-kb allele expressed higher levels of lymphotoxin than individuals homozygous for the 10.5-kb allele. Medcraft et al. (1993) studied the lymphotoxin gene polymorphism in two types of immune-complex-mediated glomerulonephritis, IgA nephropathy (IgAN) and idiopathic membranous glomerulonephritis (IMN). Their results suggest an asso-

ciation of lymphotoxin gene polymorphism with IMN and IgAN. This study also confirmed the association of the polymorphism with insulin-dependent diabetes mellitus (Ilonen et al., 1992).

No association could be established with either of the polymorphic forms in ankylosing spondylitis (Verjans et al., 1991). It is uncertain whether there is any correlation between the polymorphism described earlier and the level of lymphotoxin or any of the disease.

In the face of antigenic challenge, humoral and cell-mediated immune responses are often mutually exclusive (Parish, 1972). This phenomenon can be attributed to the cytokines that are produced upon antigenic challenge. Th1-cell responses, as a result of the production of lymphotoxin, IL-2, and IFN-γ, result in inflammatory type effector function (Mosmann and Coffman 1989). These are essential for the clearance of intracellular pathogens, but they may also account for the pathology seen during transplantation of foreign organs and cell-mediated autoimmune diseases (Powrie and Coffman 1993). In experimental autoimmune encephalomyelitis (EAE), a disease of the central nervous system mediated by T cells, the Th1 cytokines, lymphotoxin, and TNF, and in some cases IFN-γ, may correlate with the encephalitogenic capacity of $CD4^+$ T cell clones reactive to myelin basic protein (MBP) or peptide of MBP (Ando et al., 1989; Sedgwick et al., 1989; Powell et al., 1990). Lymphotoxin and TNF production is seen in the brain but not in the spinal cord, whereas IL-2, IFN-γ, and IL-4 are seen in the brain and spinal cord (Merril et al., 1992). These findings correlate with the ability of anti-TNF/lymphotoxin antibodies to prevent transfer of EAE by encephalitogenic clones (Ruddle et al., 1990).

There is evidence to suggest that lymphotoxin and TNF are both involved in multiple sclerosis (MS). In histologic analysis TNF and lymphotoxin were both detected in MS lesions but in different cells (Selmaj et al., 1991a), and Rieckmann et al. (1994) reported that the levels of lymphotoxin and TNF mRNA were higher in patients with relapsing multiple sclerosis. Lymphotoxin has been reported to be a considerably more potent killer of oligodendrocyte than TNF (Selmaj et al., 1991b). A possible role for lymphotoxin in experimental autoimmune uneoretinitis (EAU) along with IL-2 and IL-4 has also been reported by Charteris and Lighman (1993). Studies of Takahashi et al. (1993) indicated that treatment with lymphotoxin and TNF modulated autoimmunity and prevented development of IDDM in BB/ww rats which may be low producers of TNF and lymphotoxin.

Besides autoimmune disorders, Patarca et al. (1994) found dysregulated expression of lymphotoxin and TNF in patients with chronic fatigue syndrome. Foss et al. (1993) found that lymphotoxin and TNF transcripts were present in all 26 cases of Hodgkin's disease used in the study. In Hodgkin's disease-derived cell lines, supernatants contained high levels of lymphotoxin and low or undetectable levels of TNF, and in Hodgkin's Reed-Sternberg (H-RS) lines, elevated levels of lymphotoxin (Hsu and Hsu, 1989) are thought to contribute to the pathophysiology of the disease. Elevated levels of lymphotoxin, TNF, and IFN-γ were detected in serum and cerebrospinal fluid of persons infected with HIV-I (Jassoy et al., 1993).

Clark et al. (1992) found an increase in lymphotoxin in the serum of patients with malaria and suggested a role for it in hypoglycemia. Lymphotoxin kills virus-infected cells more effectively than normal cells (Aderka et al., 1985) and synergizes in several activities with interferon (Stone-Wolff et al., 1984; Williamson et al., 1983; Williams and Bellanti 1984; Pujol-Barrell et al., 1987; Trinchieri et al., 1986). Also viral infection can induce lymphotoxin production from PBL (Wong and Goeddel, 1986). Whether lymphotoxin plays any role in bacterial or fungal infections has not been reported.

XIV. CONCLUSION

There have been some very highly significant developments in the study of lymphotoxin during the last 10 years. This includes identification of its structure, isolation of both the protein and the gene, its novel role *in vivo*, and its unique interaction with certain cell surface molecules. Various studies have also indicated that lymphotoxin displays some activities that overlap with TNF and others that are quite unique to lymphotoxin. However, the molecular basis for these difference remains to be understood.

ABBREVIATIONS

TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; CSF, colony-stimulating factor; TGF, transforming growth factor; EBV, Epstein barr virus; LAK, lymphokine activated killer cells; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; RFLP, restriction fragment length polymorphism; CTLL, cytotoxic T lymphocytes.

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REFERENCES

Abe, Y., Masazumi, M., Osuka, Y., Kimura, S., Granger, G. A., & Gatanaga, T. (1992). Transforming growth factor-β_1 down-regulates expression of membrane-associated lymphotoxin and secretion of soluble lymphotoxin by human lymphokine-activated killer T cells *in vitro*. Lymphokine Cytokine Res. 11(5), 245-251.

Abe, Y., Van Eden, M., Gatanaga, M., Wang, F. I., Brightbill, H. D., Granger, G. A., & Gatanaga, T. (1993). The role of lymphotoxin in the IL-2-driven differentiation of human lymphokine-activated T-killer (T-LAK) cells *in vitro*. Lymphokine Cytokine Res. 12(5), 279-283.

Abraham, L. J., Du, D. C., Zahedi, K., Dawkins, R. L., & Whitehead, A. S. (1991). Haplotypic polymorphisms of the TNF-β gene. Immunogenetics 33, 50-53.

Aderka, D., Novick, D., Hahn, T., Fischer, D. G., & Wallach, D. (1985). Increase of vulnerability to lymphotoxin in cells infected by vesicular stomatitis virus and its further augmentation by inteferon. Cell Immunol. 92, 918-925.

Aggarwal, B. B., Moffat, B., Lee, S. H., & Harkins, R. N. (1984c). Chemical and biological properties of human lymphotoxin. In: *Thymic Hormones and Lymphokine* (Goldstein, A. L., Ed.). Plenum Publishing Corporation, New York, pp. 235-245.

Aggarwal, B. B. (1985). Human lumphotoxin. In: *Methods in Enzymology* (DiSabato, G., Ed.). Academic Press, New York, pp. 441-448.

Aggarwal, B. B., & Eessalu, T. E. (1987a). Induction of receptors for tumor necrosis factor-a by interferons is not a major mechanism for their synergistic cytotoxic response. J. Biol. Chem. 262(21), 10000-10007.

Aggarwal, B. B., & Eessalu, T. E. (1987b). Effect of phorbol esters on down-regulation and redistribution of cell surface receptors for tumor necrosis factor-α. J. Biol. Chem. 262(34), 16450-16455.

Aggarwal, B. B., & Pandita, R. (1994). Both type I and type II interferons downregulate human tumor necrosis factor receptors in human hepatocellular carcinoma cell line HEP G2: role of protein kinase C. FEBS Lett. 337, 99-102.

Aggarwal, B. B., & Vilcek, J. (Eds.) (1992). *Tumor Necrosis Factor: Structure, Function and Mechanism of Action.* Marcel Dekker, New York, pp. 1-600.

Aggarwal, B. B., Moffat, B., & Harkins, R. N. (1983b). Purification and characterization of lymphotoxin from human lymphoblastoid cell line 1788. In: *Interleukins, Lymphokines and Cytokines* (Oppenheim, J. J., Cohen, S., & Landy, M., Eds.). Academic Press, New York, pp. 521-526.

Aggarwal, B. B., Moffat, B., & Harkins, R. N. (1983a). In: *Interleukins, Lymphokines and Cytokines* (Oppenheim, J. J., Cohen, S., & Landy, M., Eds.). Academic Press, New York, pp. 521-526.

Aggarwal, B. B., Moffat., B., Lee, S. H., & Harkins, R. N. (1984a). Chemical and biological properties of human lymphotoxin. In: *Thymic Hormones and Lymphokine* (Goldstein, A. L., Ed.). Plenum Publishing Corporation, New York, pp. 235-245.

Aggarwal, B. B., Moffat, B., & Harkins, R. N. (1984b). Human lymphotoxin: Production by a lymphoblastoid cell line, purification and initial characterization. J. Biol. Chem. 259(1), 686-691.

Aggarwal, B. B., Henzel, W. J., Moffat, B., Kohr, W. J., & Harkins, R. N. (1985a). Primary structure of human lymphotoxin derived from 1788 lymphoblastoid cell line. J. Biol. Chem. 260(4), 2334-2344.

Aggarwal, B. B., Kohr, W. J., Hass, P. E., Moffat, B., Spencer, S. A., Henzel, W. J., Bringman, T. S., Nedwin, G. E., Goeddel, D. V., & Harkins, R. N. (1985b). Human tumor necrosis factor: Production, purification and characterization. J. Biol. Chem. 260(4), 2345-2354.

Aggarwal, B. B., Eessalu, T. E., & Hass, P. E. (1985c). Characterization of receptors for human tumor necrosis factor and their regulation by γ-interferon. Nature 318(6047), 665-667.

Aggarwal, B. B., Traquina, P. R., & Eessalu, T. E. (1986). Modulation of receptors and cytotoxic response of tumor necrosis factor-a by various lectins. J. Biol. Chem. 261(29), 13652-13656.

Akashi, M., Saito, M., & Koeffler, H. P. (1989). Lymphotoxin: Stimulation and regulation of colony-stimulating factors in fibroblasts. Blood 74(7), 2383-2390.

Akashi, M., Loussararian, A. H., Adelman, D. C., Saito, M., & Koeffler, H. P. (1990). Role of lymphotoxin in expression of interleukin 6 in human fibroblasts. J. Clin. Invest. 85, 121-129.

Alderson, M. R., Smith, C. A., Tough, T. W., Davis-Smith, T., Armitage, R. J., Falk, B., Roux, E., Baker, E., Sutherland, G. R., Din, W. S., & Goodwin, R. (1994). Molecular and biological characterization of human 4-1BB and its ligand. Eur. J. Immunol. 24(9), 2219-2227.

Andersson, U., Adolf, G., Dohlsten, M., Moller, G., & Sjogren, H-O. (1989). Characterization of individual tumor necrosis factor α- and β-producing cells after polyclonal T cell activation. J. Immunol. Methods 123, 233-240.

Ando, D. G., Clayton, J., Kong, D., Urban, J. L., & Sercarz, J. L. (1989). Encephaligenic T cells in the B10.PL model of experimental allergic encephalomyelitis (EAE) are of the Th-1 lymphokine subtype. Cell Immunol. 124, 132-143.

Andrews, J. S., Berger, A. E., & Ware, C. F. (1990). Characterization of the receptor for tumor necrosis factor (TNF) and lymphotoxin (LT) on human T lymphocytes: TNF and LT differ in their receptor binding properties and the induction of MHC class I proteins on a human $CD4^+$ T cell hybridoma. J. Immunol. 144(7), 2582-2591.

Androlewicz, M. J., Browning, J. L., & Ware, C. F. (1992). Lymphotoxin is expressed as a heteromeric complex with a distinct 33 kDa glycoprotein on the surface of an activated human T-cell hybridoma. J. Biol. Chem. 267, 2542-2547.

Armitage, R. J., Fanslow, W. C., Strockbine, L., Sato, T. A., Clifford, K. N., Macduff, B. M., Anderson, D. M., Gimpel, S. D., Davis-Smith, T., Maliszewski, C. R., Clark, E. A., Smith, C. A., Grabstein, K. H., Cosman, D., & Spriggs, M. K. (1992).

Molecular and biological characterization of a murine ligand for CD40. Nature 357, 80-82.

Baldwin, R. L., Mirzabekov, T., Kagan, B. L., & Wisnieski, B. J. (1995). Conformation and ion channel activity of lymphotoxin at neutral and low pH1. J. Immunol. 154, 790-798.

Banner, D. W., D'Arcy, A., Janes, W., Gentz, R., Schoenfeld, H.-J., Broger, C., Loetscher, H., & Lesslauer, W. (1993). Crystal structure of the soluble human 55 kd TNF receptor-human TNFβ complex: Implications for TNF receptor activation. Cell 73, 431-445.

Baum, P. R., Gayle, R. B., III, Ramsdell, F., Srinivasan, S., Sorensen, R. A., Watson, M. L., Seldin, M. F., Baker, E., Sutherland, G. R., Clifford, K. N., Alderson, M. R., Goodwin, R. G., & Fanslow, W. C. (1994). Molecular characterization of murine and human OX40/OX40 ligand systems: Identification of a human OX40 ligand as the HTLV-1-regulated protein gp34. EMBO J. 13(17), 3992-4001.

Beran, M., Anderson, B. S., Kelleher, P., Whalen, K., McCredie, K., & Gutterman, G. (1987). Diversity of the effect of recombinant tumor necrosis factor-α and -β on human myelogenous leukemia cell lines. Blood 69(3), 721-726.

Bersani, L., Colotta, F., Peri, G., & Mantovani, A. (1987). Cytotoxic effector function of B lymphoblasts. J. Immunol. 139, 645.

Bertolini, D. R., Nedwin, G. E., Bringman, T. S., & Mundy, G. R. (1986). Stimulation of bone resportion and inhibition of bone formation *in vitro* by human tumour necrosis factors. Nature 319, 516.

Beutler, B., Mahoney, J., Le Trang, N., Pekala, P., & Cerami, A. (1985). Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. J. Exp. Med. 161, 984-995.

Brach, M. A., Cicco, N. A., Riedel, D., Hirano, T., Kishimoto, T., Mertelsmann, R. H., & Herrmann, F. (1990). Mechanisms of differential regulation of interleukin-6 mRNA accumulation by tumor necrosis factor alpha and lymphotoxin during monocytic differentiation. FEBS Lett. 263(2), 349-354.

Brakebusch, C., Nophar, Y., Kemper, O., Engelman, H., & Wallach, D. (1992). Cytoplasmic truncation of the p55 tumour necrosis factor (TNF) receptor abolishes signalling, but not induced shedding of the receptor. EMBO J. 11, 943-950.

Brockhaus, M., Schoenfeld, H.-J., Schlaeger, E.-J., Hunziker, W., Lesslauer, W., & Loetscher, H. (1990). Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc. Natl. Acad. Sci. USA 87, 3127-3131.

Broudy, V. C., Harlan, J. M., & Adamson, J. W. (1987). Disparate effects of tumor necrosis factor-α/cachectin and tumor necrosis factor-β/lymphotoxin on hematopoietic growth factor production and neutrophil adhesion molecule expression by cultured human endothelial cells. J. Immunol. 138(12), 4298-4302.

Browning, J., & Ribolini, A. (1989). Studies on the differing effects of tumor necrosis factor and lymphotoxin on the growth of several human tumor lines. J. Immunol. 143(6), 1859-1867.

Browning, J. L., Androlewicz, M. J., & Ware, C. F. (1991). Lymphotoxin and an associated 33 kDa glycoprotein are expressed on the surface of an activated human T cell hybridoma. J. Immunol. 147, 1230-1237.

Browning, J. L., Ngam-ek, A., Lawton, P., DeMarinis, J., Tizard, R., Chow, E. P., Hession, C., O'Brine-Greco, B., Foley, S. F., & Ware, C. F. (1993). Lymphotoxin b, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. Cell 72, 847-856.

Browning, J. L., Dougas, I., Ngam-ek, A., Bourdon, P. R., Ehrenfels, B. N., Miatkowski, K., Zafari, M., Yampaglia, A. M., Lawton, P., Meier, W., Benjamin, C. P., & Hession, C. (1995). Characterization of surface lymphotoxin forms. J. Immunol. 154, 33-46.

Buck, C., Digel, W., Schoniger, W., Stefanic, M., Raghavachar, A., Heimpel, H., & Porzsolt, F. (1990). Tumor necrosis factor-α, but not lymphotoxin, stimulates growth of tumor cells in hairy cell leukemia. Leukemia 4(6), 431-434.

Buonaguro, L., Barillari, G., Chang, H. K., Bohan, C. A., Kao, V., Morgan, R., Gallo, R. C., & Ensoli, B. (1992). Effects of the human immunodeficiency virus type 1 Tat protein on the expression of inflammatory cytokines. J. Virol. 66, 7159-7167.

Buonaguro, L., Buonaguro, F. M., Giraldo, G., & Ensoli, B. (1994). The human immunodeficiency virus type I tat protein transactivates tumor necrosis factor beta gene expression through a TAR-Like structure. J. Virol. 68(4), 2677-2682.

Camerini, D., Walz, G., Loenen, W. A., Borst, J., & Seed, B. (1991). The T cell activation antigen CD27 is a member of the nerve growth factor/tumor necrosis factor receptor gene family. J. Immunol. 147, 3165-3169.

Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown, S. S., & Cerami, A. (1986). Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. Natl. Acad. Sci. U. S. A. 83, 1670.

Chan, H., & Aggarwal, B. B. (1994). Role of tumor necrosis factor receptors in the activation of nuclear factor κB in human histiocytic lymphoma U-937 cells. J. Biol. Chem. 269(50), 31424-31429.

Charteris, D. G., & Lightman, S. L. (1993). *In vivo* lymphokine production in experimental autoimmune uveoretinities. Immunology 78(3), 387-392.

Chatterjee, S. (1994). Neutral sphingomyelinase action stimulates signal transduction of tumor necrosis factor-alpha in the synthesis of cholesteryl ester in human fibroblasts. J. Biol. Chem. 269(2), 879-882.

Chaturvedi, M., LaPushin, R., & Aggarwal, B. B. (1994). Tumor necrosis factor and lymphotoxin: qualitative and quantitative differences in the mediation of early and late cellular responses. J. Biol. Chem. 269(20), 14575-14583.

Cicco, N. A., Lindemann, A., Content, J., Vandenbussche, P., Lübbert, M., Gauss, J., Mertelsmann, R., & Hedrrmann, F. (1990). Inducible production of interleukin-6 by human polymorphonuclear neutrophils: Role of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha. Blood 75(10), 2049-2052.

Clark, I. A., Gray, K. M., Rockett, E. J., Cowden, W. B., Rockett, K. A., Ferrante, A., & Aggarwal, B. B. (1992). Increased lymphotoxin in human malarial serum and the ability of this cytokine to increase plasma IL-6 and cause hypoglycaemia in mice: implications for malarial pathology. Trans. R. Soc. Trop. Med. Hyg. 86, 602-607.

Collart, M. A., Baeuerle, P., & Vassalli, P. (1990). Regulation of tumor necrosis factor alpha transcription in macrophages: Involvement of four kB-like motifs and constitutive and inducible forms of NF-kB. Mol. Cell. Biol. 110, 1498-1506.

Crowe, P. D., VanArsdale, T. L., Walter, B. N., Dahms, K. M., & Ware, C. F. (1994a). Production of lymphotoxin (LTα) and a soluble dimeric form of its receptor using the baculovirus expression system. J. Immunol. Methods 168, 79-89.

Crowe, P. D., Vanarsdale, T. L., Walter, B. N., Ware, C. F., Hession, C., Ehrenfels, B., Browning, J. L., Din, W., Goodwin, R. G., & Smith, C. A. (1994b). A lymphotoxin-β specific receptor. Science 264, 707-710.

Cuturi, M. C., Murphy, M., Costa-Giomi, M. P., Weinmann, R., Perussia, B., & Trinchieri, G. (1987). Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. J. Exp. Med. 165, 1581-1594.

Darnay, B. G., Reddy, S. A. G., & Aggarwal, B. B. (1994a). Identification of a protein kinase associated with the cytoplasmic domain of the p60 tumor necrosis factor receptor. J. Biol. Chem. 269(32), 20299-20304.

Darnay, B. G., Reddy, S. A. G., & Aggarwal, B. B. (1994b). Physical and functional association of a serine-threonine protein kinase to the cytoplasmic domain of the p80 form of the human tumor necrosis factor receptor in human histiocytic lymphoma U-937 cells. J. Biol. Chem. 269(31), 19687-19690.

De Togni, P., Goellner, J., Ruddle, N. H., Streeter, P. R., Fick, A., Mariathasan, S., Smith, S. C., Carlson, R., Shornick, L. P., Strauss-Schoenberger, J., Russell, J. H., Karr, R., & Chaplin, D. D. (1994). Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science 264, 703-707.

Desch, C. E., Dobrina, A., Aggarwal, B. B., & Harlan, J. M. (1990). Tumor necrosis factor-α exhibits greater proinflammatory activity than lymphotoxin *in vitro*. Blood 75(10), 2030-2034.

Dett, C. A., Gatanaga, M., Ininns, E. K., Cappuccini, F., Yamamoto, R. S., Granger, G. A., & Gatanaga, T. (1991). Enhancement of lymphokine-activated T killer cell tumor necrosis factor receptor mRNA transcription. Tumor necrosis factor receptor membrane expression, and tumor necrosis factor/lymphotoxin release by IL-1b, IL-4, and IL-6 *in vitro*. J. Immunol. 146(5), 1522-1526.

Ding, A. H., Sanchez, E., Srimal, S., & Nathan, C. F. (1989). Macrophage rapidly internalize their tumor necrosis factor receptors in response to bacterial lipopolysaccharide. J. Biol. Chem. 264, 3924-3935.

Dürkop, H., Latza, U., Hummel, M., Eitelbach, F., Seed, B., & Stein, H. (1992). Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell 68, 421-427.

Eck, M. J., & Sprang, S. R. (1989). The stucture of tumor necrosis factor-β at 2.6 Å resolution. J. Biol. Chem. 264, 17595-17605.

English, B. K., Weaver, W. M., & Wilson, C. B. (1991). Differential regulation of lymphotoxin and tumor necrosis factor genes in human T lymphocytes. J. Biol. Chem. 266(11), 7108-7113.

Erickson, S. L., de Sauvage, F. J., Kikly, K., Carver-Moore, K., Pitts-Meek, S., Gillett, N., Sheehan, K. C. F., Schrelber, R. D., Goeddel, D. V., & Moore, M. W. (1994). Decreased sensitivity to tumour-necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. Nature 372, 560.

Espevik, T., Figari, I. S., Shalaby, M. R., Lackides, G. A., Lewis, G. D., Shepard, H. M., & Palladino, M. A., Jr. (1987). Inhibition of cytokine production by cyclosporin A and transforming growth factor b. J. Exp. Med. 166, 571-576.

Espevik, T., Brockhaus, M., Loetscher, H., Nonstad, U., & Shalaby, R. (1990). Characterization of binding and biological effects of monoclonal antibodies against a human tumor necrosis factor receptor. J. Exp. Med. 171, 415-426.

Estrov, Z., Kurzrock, R., Pocsik, E., Pathak, S., Kantarjian, H. M., Zipf, T. F., Harris, D., Talpaz, M., & Aggarwal, B. B. (1993). Lymphotoxin is an autocrine growth factor for Epstein-Barr virus (EBV)-infected B- cell lines. J. Exp. Med. 177, 763-774.

Eugster, H. P. (1994). TNFα and LTα double knockout mice: Definitive answers to old questions? Eur. Cytokine Netw. 5(2), 146.

Fashena, S. J., Tang, W.-L., Sarr, T., & Ruddle, N. H. (1990). The murine lymphotoxin gene promoter: characterization and negative regulation. J. Immunol. 145(1), 177-183.

Fashena, S. J., Reeves, R., & Ruddle, N. H. (1992). Upstream activating sequence binds high mobility group I protein and contributes to lymphotoxin (tumor necrosis factor-β) gene regulation. Mol. Cell. Biol. 12, 894-903.

Ferrante, A., Nandoskar, M., Bates, E. J., Goh, H. B., & Beard, L. J. (1988). Tumor necrosis factor beta (lymphotoxin) inhibits locomotion and stimulates the respiratory burst and degranulation of neutrophils. Immunology 63, 507.

Ferreri, N. R., Sarr, T., Askenase, P. W., & Ruddle, N. H. (1992). Molecular regulation of tumor necrosis factor-α and lymphotoxin production in T cells: Inhibition by prostaglandin E2. J. Biol. Chem. 267, 9443.

Figari, I. S., Mori, N. A., & Palladino, M. A., Jr. (1987). Regulation of neutrophil migration and superoxide production by recombinant tumor necrosis factors-alpha and -beta: Comparison to recombinant interferon-gamma and interleukin-1alpha. Blood 70, 979.

Fischer, H., Dohlstein, M., Andersson, U., Hedlund, G., Ericsson, P., Hansson, J., & Sjogren, H. O. (1990). Production of TNFα and TNFβ by staphylococcal enterotoxin A activated human T cells. J. Immunol. 144, 4663-4669.

Foss, H. D., Herbst, H., Oelmann, E., Samol, J., Grebe, M., Blankenstein, T., Matthes, J., Quin, Z. H., Falini, B., Pileri, S., Diamantstein, T., & Stein, H. (1993). Lymphotoxin, tumour necrosis factor and interleukin-6 gene transcripts are present in Hodgkin and Reed-Sternberg cells of most Hodgkin's disease cases. Br. J. Haematol. 84(4), 627-635.

Frohman, E. M., Frohman, T. C., Dustin, M. L., Vayuvegula, B. C., Gupta, A., van den Noort, S., & Gupta, S. (1989). The induction of intercellular adhesion molecule 1 (ICAM-1) expression on human fetal astrocytes by interferon-γ, tumor necrosis factor α, lymphotoxin, and interleukin-1: Relevance to intracerebral antigen presentation. J. Neuroimmunol. 23, 117-124.

Fuhrer, J. P., & Evans, C. H. (1982). Rapid separation of biologically active Syrian hamster lymphotoxin in high yield by size-exclusion high-performance liquid chromatography. J. Chromatogr. 248(3), 427-433.

Fukushima, K., Watanabe, H., Takeo, K., Nomura, M., Asahi, T., & Yamashita, K. (1993). N-linked sugar chain structure of recombinant human lymphotoxin produced by CHO cells: The functional role of carbohydrate as to its lectin-like character and clearance velocity. Arch. Biochem. Biophys. 304(1), 144-153.

Funahashi, I., Watanabe, H., Abo, T., Indo, K., & Miyaji, H. (1993). Tumour growth inhibition in mice by glycosylated recombinant human lymphotoxin: Analysis of tumour-regional mononuclear cells involved with its action. Br. J. Cancer 67, 447-455.

Gardner, S. M., Mock, B. A., Hilgers, J., Huppi, K. E., & Roeder, W. D. (1987). Mouse lymphotoxin and tumor necrosis factor: Structural analysis of the cloned genes, physical linkage and chromosomal position. J. Immunol. 139, 476-483.

Garrett, I. R., Durie, B. G. M., Nedwin, G. E., Gillespie, A., Bringman, T., Sabatini, M., Bertolini, D. R., & Mundy, G. R. (1987). Production of the bone resorbing cytokine lymphotoxin by cultured human myeloma cells. N. Engl. J. Med. 317, 526.

Goeddel, D. V., Aggarwal, B. B., Gray, P. W., Leung, D. W., Nedwin, G. E., Palladino, M. A., Patton, J. S., Pennica, D., Shepard, H. M., Sugarman, B. J., & Wong, G. H. W. (1986). Tumor necrosis factors: Gene structure and biological activities. Cold Spring Harbor Symposia on Quantitative Biology L1, 597-609.

Goodwin, R. G., Anderson, D., Jerzy, R., Davis, T., Brannan, C. I., Copeland, N. G., Jenkins, N. A., & Smith, C. A. (1991). Molecular cloning and expression of the type 1 and type 2 murine receptors for tumor necrosis factor. Mol. Cell. Biol. 11(6), 3020-3026.

Goodwin, R. G., Din, W. S., Davis-Smith, T., Anderson, D. M., Gimpel, S. D., Maliszewski, C. R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., Farrah, T., Armitage, R. J., Fanslow, W. C., & Smith, C. A. (1993). Molecular cloning of a ligand for the inducible T cell gene 4-1BB: A member of an emerging family of cytokines with homology to tumor necrosis factor. Eur. J. Immunol. 23, 2631-2641.

Govaerts, A. (1960). Cellular antibodies in kidney homotransplantation. J. Immunol. 85, 516-522.

Granger, G. A., & Williams, T. W. (1968). Lymphocyte cytotoxicity *in vitro*: Activation and release of a cytotoxic factor. Nature 218, 1253-1254.

Gravestein, L. A., Blom, B., Nolten, L. A., de Vries, E., van der Horst, G., Ossendorp, F., Borst, J., & Loenen, W. A. (1993). Cloning and expression of murine CD27: comparison with 4-1BB, another lymphocyte-specific member of the nerve growth factor receptor family. Eur. J. Immunol. 23, 943-950.

Gray, P. W., Aggarwal, B. B., Benton, C. V., Bringman, T. S., Henzel, W. J., Jarrett, J. A., Leung, D. W., Moffat, B., Ng, P., Svedersky, L. P., Palladino, M. A., & Nedwin, G. A. (1984). Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumor necrosis activity. Nature 312(5996), 721-724.

Gullberg, U., & Nilsson, E. (1989). Recombinant lymphotoxin enhances the growth of normal, but not of chronic myeloid leukemic, human hematopoietic progenitor cells *in vitro*. Leuk. Res. 13(11), 953-957.

Hains, J. M., & Aggarwal, B. B. (1989). Characterization of recombinant human lymphotoxin (TNF-β) produced by a mammalian cell line. Arch. Biochem. Biophys. 274(2), 417-425.

Haas, P. E., Hotchkiss, A., Mohler, M., & Aggarwal, B. B. (1985). Characterization of specific high affinity receptors for human tumor necrosis factor on mouse fibroblasts. J. Biol. Chem. 260(22), 12214-12218.

Heller, R. A., Song, K., Onasch, M. A., Fischer, W. H., Chang, D., & Ringold, G. M. (1990). Complementary DNA cloning of a receptor for tumor necrosis factor and demonstration of a shed form of the receptor. Proc. Nat. Acad. Sc. USA 87, 6151-6155.

Hemmi, H., Nakamura, T., Tamura, K., Shimizu, Y., Kato, S., Miki, T., Takahashi, N., Muramatsu, M., Numao, N., & Sugarman, K. (1987). Lymphotoxin: Induction of terminal differentiation of the human myeloid leukemia cell lines HL-60 and THP-1. J. Immunol. 138(3), 664-666.

Higuchi, M., & Aggarwal, B. B. (1993). Okadaic acid induces downmodulation and shedding of tumor necrosis factor receptors: Comparison with another tumor promoter, phorbol ester. J. Biol. Chem. 268(8), 5624-5631.

Higuchi, M., & Aggarwal, B. B. (1994). Tumor necrosis factor induces internalization of the p60 receptor and shedding of the p80 receptor. J. Immunol. 152(7), 3550-3558.

Himmler, A., Maurer-Fogy, I., Kronke, M., Scheurich, P., Pfizenmaier, K., Lantz, M., Olsson, I., Hauptmann, R., Stratowa, C., & Adolf, G. R. (1990). Molecular cloning and expression of human and rat tumor necrosis factor receptor chain (p60) and its soluble derivative, tumor necrosis factor-binding protein. DNA Cell Biol. 9(10), 705-715.

Hirahara, H., Ogawa, M., Kimura, M., Iiai, T., Tsuchida, M., Hanawa, H., Watanabe, H., & Abo, T. (1994). Glucocorticoid independence of acute thymic involution induced by lymphotoxin and estrogen. Cell Immunol. 153, 401-411.

Hirano, K., Yamamoto, K., Kobayashi, Y., & Osawa, T. (1989). Characterization of specific high-affinity receptor for human lymphotoxin. J. Biochem. 105, 120-126.

Hofsli, E., Austgulen, R., & Nissen-Meyer, J. (1987). Lymphotoxin-induced growth stimulation of diploid human fibroblasts in the presence and absence of gamma interferon. Scand. J. Immunol. 26, 585-588.

Hohmann, H.-P., Remy, R., Poschl, B., & van Loon, A. P. G. M. (1990). Tumor necrosis factors-a and -b bind to the same two types of tumor necrosis factor receptors and maximally activate the transcription factor NF-κB at low receptor occupancy and within minutes after receptor binding. J. Biol. Chem. 265(25), 15183-15188.

Hohmann, H. P., Remy, R., Brockhaus, M., & van Loon, A. P. G. M. (1989). Two different cell types have different major receptors for human tumor necrosis factor (TNF-α). J. Biol. Chem. 264, 14927-14934.

Holt, S. J., Grimble, R. F., & York, D. A. (1989). Tumour necrosis factor-α and lymphotoxin have opposite effects on sympathetic efferent nerves to brown adipose tissue by direct action in the central nervous system. Brain Res. 497, 183-186.

Holtmann, F., & Wallach, D. (1987). Downregulation of the receptors for tumor necrosis factor by interleukin-1 and phorbol ester. J. Immunol. 139, 1161-1167.

Hsu, P-L., & Hsu, S-M. (1989). Production of tumor necrosis factor-α and lymphotoxin by cells of Hodgkin's neoplastic cell lines HDLM-1 and KM-H2. Am. J. Pathol. 135(4), 735-745.

Hu, F.-Q., Smith, C. A., & Pickup, D. J. (1994). Cowpox virus contains two copies of an early gene encoding a soluble secreted form of the type II TNF receptor. Virology 204, 343-356.

Ilonen, J., Merivuori, H., Reijonen, H., Knip, M., Akerblom, H. K., Pociot, F., & Nerup, J. (1992). Tumour necrosis factor-β gene RFLP alleles in finnish IDDM haplotypes. Scand. J. Immunol. 36, 779-783.

Ishibashi, K., Ishitsuka, K., Chuman, Y., Otsuka, M., Kuwazuru, Y., Iwahashi, M., Utsunonnya, A., Handa, S., Sakurami, T., & Arima, T. (1991). Tumor necrosis factor-β in the serum of adult T-cell leukemia with hypercalcemia. Blood 77, 2451-2455.

Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S., Sameshima, M., Hase, A., Seto, Y., & Nagata, S. (1991). The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 66(2), 233-243.

Itoh, N., Tsujimoto, Y., & Nagata, S. (1993). Effect of bcl-2 on fas antigen-mediated cell death. J. Immunol. 151(2), 621-627.

Iwamoto, S., Shibuya, I., Takeda, K., & Takeda, M. (1994). Lymphotoxin lacks effects on 75-kDa receptors in cytotoxicity on U-937 cells. Biochem. Biophys. Res. Commun. 199(1), 70-77.

Jassoy, C., Harrer, T., Rosenthal, T., Navia, B. A., Worth, J., Johnson, R. P., & Walker, B. D. (1993). Human immunodeficiency virus type 1-specific cytotoxic T lymphocytes release gamma interferon, tumor necrosis factor α (TNF-α), and TNF-beta when they encounter their target antigens. J. Virol. 67(5), 2844-2852.

Johnson, D., Lanahan, A., Buck, C. R., Sehgal, A., Morgan, C., Mercer, E., Bothwell, M., & Chao, M. (1986). Expression and structure of the human NGF receptor. Cell 47, 545-554.

Jongeneel, C. V., Shakhov, A. N., Nedospasov, S. A., & Cerotini, J-C. (1989). Molecular control of tissue-specific expression at the mouse TNF locus. Eur. J. Immunol. 19, 549.

Jongeneel, C. V., Griant, L., Udalovo, I. A., Sevin, A., Nedospasov, S. A., & Cambon-Thomsen, A. (1991). Extensive genetic polymorphism in the human tumor necrosis factor region and relation to extended HLA haplotypes. Proc. Natl. Acad. Sci. USA 88(21), 9717-9721.

Kapp, A., Zeck-Kapp, G., & Blohm, D. (1989). Human tumor necrosis factor is a potent activator of the oxidative metabolism in human polymorphonuclear neutrophilic granulocytes: Comparison with human lymphotoxin. J. Invest. Dermatol. 92(3), 348-354.

Kasid, A., Director, E. P., Stovroff, M. C., Lotze, M. T., & Rosenberg, S. A. (1990). Cytokine regulation of tumor necrosis factor-α and -β (lymphotoxin)-messenger RNA expression in human peripheral blood mononuclear cells. Cancer Res. 50, 5072-5076.

Kato, S., Miki, T., Takahashi, N., Ohmori, M., Hemmi, H., Numao, N., & Kondo, K. (1989). Lymphotoxin cDNA clones from a HTLV-I-carrying T cell line HUT-102. AIDS Res. Hum. Retroviruses 5(6), 663-670.

Kaushansky, K., Broudy, V. C., Harlan, J. M., & Adamson, J. W. (1988). Tumor necrosis factor-α and tumor necrosis factor-β (lymphotoxin) stimulate the production of granulocyte-macrophage colony-stimulating factor, macrophage colony stimulating factor, and IL-1 *in vivo*. J. Immunol. 141(10), 3410-3415.

Kawatsu, M., Funahashi, I., Kajikawa, T., Takeo, K., Asahi, T., Yamashita, T., Kawaharada, H., & Watanabe, K. (1990a). Synergistic antitumor effect of glycosylated recombinant human lymphotoxin with human interferon-γ on lymphotoxin- sensitive human tumor. J. Interferon Res. 10, 519-529.

Kawatsu, M., Takeo, K., Kajikawa, T., Funahashi, I., Ashahi, T., Kakutani, T., Yamashita, T., Kawaharada, H., & Watanabe, K. (1990b). The pharmacokinetic pattern of glycosylated human recombinant lymphotoxin (LT) in rats after intravenous administration. J. Pharmacobiodyn. 13, 549-557.

Kehrl, J. H., Alvarez-Mon, M., Delsing, G. A., & Fauci, A. S. (1987). Lymphotoxin is an important T cell-derived growth factor for human B cells. Science 238, 1144.

Kircheis, R., Milleck, J., Korobko, V. G., Shingarova, L. N., & Schmidt, H. E. (1992). Differences in the biological activity of TNFα and TNFβ correlate with their different abilities for binding to the target cell. Eur. Cytokine Netw. 3(4), 381-390.

Kobayashi, Y., Miyamotu, D., Asada, M., Obinata, M., & Osawa, T. (1986). Cloning and expression of human lymphotoxin mRNA derived from a human T cell hybridoma. J. Biochem. 100, 727-733.

Koeffler, H. P., Gasson, J., Ranyard, J., Souza, L., Shepard, M., & Munker, R. (1987). Recombinant human TNF-α stimulates production of granulocyte colony-stimulating factor. Blood 70(1), 55-59.

Kohno, T., Brewer, M. T., Baker, S. L., Schwartz, P. E., King, M. W., Hal, K. K., Squires, C. H., Thompson, R. C., & Vannice, J. (1990). A second tumor necrosis factor receptor gene product can shed a naturally occurring tumor necrosis factor inhibitor. Proc. Natl. Acad. Sci. USA 87, 8331-8335.

Kownatzki, E., Kapp, A., & Uhrich, S. (1988). Modulation of human neutrophilic granulocyte functions by recombinant human tumor necrosis factor and recombinant human lymphotoxin. Promotion of adherence, inhibition of chemotactic migration and superoxide anion release from adherent cells. Clin. Exp. Immunol. 74(1), 143-148.

Kramer, S. M., Carver, M. E., & Apperson, S. M. (1986). Comparison of TNF-α and TNF-β cytolytic biological activities in a serum free bioassay. Lymphokine Res. 5, S139.

Kreil, E. A., Greene, E., Fitzgibbon, C., Robinson, D. R., & Zapol, W. M. (1989). Effects of recombinant human tumor necrosis factor alpha, lymphotoxin, and *Escherichia coli* lipopolysaccharide on hemodynamics, lung microvascular permeability, and eicosanoid synthesis in anesthetized sheep. Circ. Res. 65(2), 502-514.

Kriegler, M., Perez, C., DeFay, K., Albert, I., & Lu, S. D. (1988). A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: Ramification for the complex physiology of TNF. Cell 53, 45.

Kronke, M., Hensel, G., Schluter, C., Scheurich, P., Schutze, S., & Pfizenmaier, K. (1988). Tumor necrosis factor and lymphotoxin gene expression in human tumor cell lines. Cancer Res. 48, 5417-5421.

Kwon, B. S. & Weissman, S. M. (1989). cDNA sequences of two inducible T-cell genes. Proc. Natl. Acad. Sci. USA 86, 1963-1967.

Laskov, R., Lancz, G., Ruddle, N. H., McGrath, K. M., Spector, S., Klein, T., Djeu, J. Y., & Friedman, H. (1990). Production of tumor necrosis factor (TNF-α) and lymphotoxin (TNF-β) by murine pre-B and B-cell lymphomas. J. Immunol. 144, 3424.

Lee, S. H., Aggarwal, B. B., Rinderknecht, E., Assisi, F., & Chiu, H. (1984). The synergistic antiproliferative effect of γ-interferon and human lymphotoxin. J. Immunol. 133(3), 1083-1086.

Lewis, M., Tartaglia, L. A., Lee, A., Bennett, G. L., Rice, G. C., Wong, G. H., Chen, E. Y., & Goeddel, D. V. (1991). Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. Proc. Natl. Acad. Sci. USA 88(7), 2830-2834.

Li, C.-B., Gray, P. W., Lin, P.-F., McGrath, K. M., Ruddle, F. H., & Ruddle, N. H. (1987). Cloning and expression of murine lymphotoxin cDNA. J. Immunol. 138(12), 4496-4501.

Lipoldova, M., Zajicova, A., & Holan, V. (1993). Expression of the gene for tumor necrosis factor-β but not for tumor necrosis factor-α is impaired in tumor-bearing mice. Cell Immunol. 152, 234-239.

Locksley, R. M., Heinzel, F. P., Shepard, H. M., Agosti, J., Eessalu, T. E., Aggarwal, B. B., & Harlan, J. M. (1987). Tumor necrosis factor-α and -β differ in their capacities to generate interleukin 1 release from human endothelial cells. J. Immunol. 139(6), 1891-1895.

Loetscher, H. Y., Pan, C. E., Lahm, H. W., Gentz, R., Brockhaus, M., Tabuchi, H., & Lesslauer, W. (1990). Molecular cloning and expression of the human 55kd tumor necrosis factor receptor. Cell 61, 351-359.

Lucas, R., Magez, S., DeLeys, R., Fransen, L., Scheerlinck, J.-P., Rampelberg, M., Sablon, E., & DeBaetselier, P. (1994). Mapping the lectin-like activity of tumor necrosis factor. Science 263, 814-817.

Mallett, S., Fossum, S., & Barclay, A. N. (1990). Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes-a molecule related to nerve growth factor receptor. EMBO J. 9(4), 1063-1068.

Mantovani, L., Henschler, R., Brach, M. A., Wieser, R., Lübbert, M., Lindemann, A., Mertelsmann, R. H., & Herrmann, F. (1990). Differential regulation of interleukin-6 expression in human fibroblasts by tumor necrosis factor-a and lymphotoxin. FEBS Lett. 270(1,2), 152-156.

Matsubara, N., Fuchimoto, S., & Orita, K. (1990). Differing roles of protein kinase C on the antiproliferative effects of tumor necrosis factor α and β on LoVo cells. Pathobiology 58, 168-171.

Matsuyama, N., Okawa, N., Tsukii, Y., Endo, T., & Kaji, A. (1992). Nucleotide sequence of a cDNA encoding human tumor necrosis factor β from B lymphoblastoid cell RPMI 1788. FEBS Lett. 302(2), 141-144.

Medcraft, J., Hitman, G. A., Sachs, J. A., Whichelow, C. E., Raafat, I., & Moore, R. H. (1993). Autoimmune renal disease and tumour necrosis factor β gene polymorphism. Clin. Nephrol. 40(2), 63-68.

Melani, C., Silvani, A., Parmiani, G., & Colombo, M. P. (1993). Lymphotoxin gene expression by melanocytes and melanoma cell lines and persistence of unspliced mRNA. FEBS Lett. 335(1), 114-118.

Merril, J. E., Kono, D. H., Clayton, J., Ando, D. G., Hinton, D. R., & Hofman, F. M. (1992). Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. Proc. Natl. Acad. Sci. USA 89, 574-578.

Messer, G., Spangler, U., Jung, M. C., Honold, G., Blomer, K., Pape, G. R., Riethmuller, G., & Weiss, E. H. (1991). Polymorphic structure of the tumor necrosis factor (TNF) locus: An *Nco* I polymorphism in the first intron of the human TNF-β production. J. Exp. Med. 173, 209-219.

Moberts, R., Hoogerbrugge, H., van Agthoven, T., Lowenberg, B., & Touw, I. (1989). Proliferative response of highly purified B chronic lymphocytic leukemia cells in serum free culture to interleukin-2 and tumor necrosis factors alpha and beta. Leuk. Res. 13(11), 973-980.

Mosmann, T. R. & Coffman, R. L. (1989). Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. 7, 145-173.

Mrowietz, U., Ternowitz, T., Schroder, J.-M., & Christophers, E. (1989). Recombinant human tumour necrosis factor β (lymphotoxin) lacks chemotactic activity for human peripheral blood neutrophils, monocytes, and T cells. Scand. J. Immunol. 30, 373-377.

Muller, U., Jongeneel, C. F., Nedospasov, S. A., Lindahl, K. F., & Steinmetz, M. (1987). Tumour necrosis factor and lymphotoxin genes map close to H-2D in the mouse major histocompatibility complex. Nature 325, 265-267.

Muller-Alouf, H., Alouf, J. E., Ozegowski, J. H., Fitting, C., & Cavaillon, J. M. (1994). Comparative study of cytokine release by human peripheral blood mononuclear cells stimulated with *Streptococcus pyogenes* superantigenic erythrogenic toxins, heat-killed streptococci, and lipopolysaccharide. Infect. Immun. 62(11), 4915-4921.

Muñoz-Fernandez, M. A., Cano, E., O'Donnell, C. A., Doyle, J., Liew, F. Y., & Fresno, M. (1994). Tumor necrosis factor-α, interferon-γ, and interleukin-6 but not TNF-β induce differentiation of neuroblastoma cells: The role of nitric oxide. J. Neurochem. 62, 1330-1336.

Murphy, M., Perussia, B., & Trinchieri, G. (1988). Effects of recombinant tumor necrosis factor, lymphotoxin, and immune interferon on proliferation and differentiation of enriched hematopoietic precursor cells. Exp. Hematol. 16, 131-138.

Nedospasov, S. A., Shakhov, A. N., Turetskaya, R. L., Mett, V. A., Georgiev, G. P., & Korobko, V. G. (1985). Molecular cloning of human tumor necrosis factor gene: Tandem arrangement of TNF-α and TNF-β genes in short segment of human genome. Dokl. Akad. Nauk. SSSR 285, 1487.

Nedospasov, S. A., Shakhov, A. N., Turetskaya, R. L., Mett, V. A., Azizov, M. M., Georgiev, G. P., Korobko, V. G., Dobrynin, V. N., Filippov, S. A., Bystrov, N. S., Boldyreva, E. F., Chuvpilo, S. A., Chumakov, A. M., Shingarova, L. N., & Ovchinnikov, Y. A (1986a). Tandem arrangement of genes coding for tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta) in the human genome. Cold Spring Harbor Symposia on Quantitative Biology 51(Pt 1), 611-624.

Nedospasov, S. A., Hirt, B., Shakhov, A. N., Dobrynin, V. N., Kawashima, E., Accolla, R. S., & Jongeneel, C. V. (1986b). The genes for tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta) are tandemly arranged on chromosome 17 of the mouse. Nucleic Acids Res. 14, 7713.

Nedospasov, S. A., Turetskaya, R. L., & Shakhov, A. N. (1988). Cloning and characterization of the rabbit lymphotoxin (TNF-β) gene and its tight linkage to the gene coding for tumor necrosis factor. Nucleic Acids Res. 16, 3112.

Nedospasov, S. A., Udalova, I. A., Kuprash, D. V., & Turetskaya, R. L. (1991). DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus. J. Immunol. 147, 1053-1059.

Nedwin, G. E., Svedersky, L. P., Bringman, T. s., Palladino, M. A., Jr., & Goeddel, D. V. (1985a). Effect of interleukin 2, interferon-gamma, and mitogens on the

production of tumor necrosis factors alpha and beta. J. Immunol. 135(4), 2492-2497.

Nedwin, G., Naylor, S. L., Sakaguchi, A. Y., Smith, D., Nedwin, G. E., Pennica, D., Goedell, D. V., & Gray, P. W. (1985b). Human lymphotoxin and tumor necrosis factor genes: Structure homology and chromosome localization. Nucleic Acids Res. 13(17), 6361-6372.

Nophar, Y., Kemper, O., Brakebusch, C., Engelman, H., Zwang, R., Aderka, D., Holtman, H., & Wallach, D. (1990). Soluble forms of tumor necrosis factor receptors (TNF-Rs). The cDNA for the type I TNF-R, cloned using amino acid sequence data of its soluble form, encodes both the cell surface and a soluble form of the receptor. J. EMBO 9, 3269-3278.

Oster, W., Lindemann, A., Horn, S., Mertelsmann, R., & Herrmann, F. (1987). Tumor necrosis factor (TNF)-α but not TNF-β induces secretion of colony stimulating factor for macrophages (CSF-1) by human monocytes. Blood 70(5), 1700-1703.

Owen-Schaub, L. B., Crump, W. L., III., Morin, G. I., & Grimm, E. A. (1989). Regulation of lymphocyte tumor necrosis factor receptors by IL-2. J. Immunol. 143, 2236.

Pang, X.-P., Hershman, J. M., Chung, M., & Pekary, E. (1989). Characterization of tumor necrosis factor-a receptors in human and rat thyroid cells and regulation of the receptors by thyrotropin. Endocrinology 125, 1783-1788.

Parish, C. R. (1972). The relationship between humoral and cell-mediated immunity. Transplant. Rev. 13, 35-66.

Partanen, J., & Koskimies, S. (1988). Low degree of DNa polymorphism in the HLA-linked lymphotoxin (tumor necrosis factor-β) gene. Scand. J. Immunol. 28, 313-316.

Patarca, R., Klimas, N. G., Lugtendorf, S., Antoni, M., & Fletcher, M. A. (1994). Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome: Interrelations with cellular sources and patterns of soluble immune mediator expression. Clin. Infect. Dis. 18(Suppl 1), S147-S153.

Patton, J. S., Shepard, H. M., Wilking, H., Lewis, G., Aggarwal, B. B., Eessalu, T. E., Gavin, L. A., & Grunfeld, C. (1986). Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. Proceedings of the National Academy of Science, USA 83, 8313-8317.

Paul, N. L., Lenardo, M. J., Novak, K. D., Sarr, T., Tang, W.-L., & Ruddle, N. H. (1990). Lymphotoxin activation by human T-cell leukemia virus type I infected cell lines: role for NK-κB. J. Virol. 64, 5412.

Pennica, D., Nedwin, G. E., Hayflick, J. F., Seeburg, P. H., Palladino, M. A., Kohr, W. J., Aggarwal, B. B., & Goeddel, D. V. (1984). Human tumor necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 312(5996), 724-729.

Pfeffer, K., Matsuyama, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Kronke, M., & Mak, T. W. (1993). Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock yet succumb to *L. monocytogenes* infection. Cell 73, 457-467.

Pfeilschifter, J., Mundy, G. R., & Roodman, G. D. (1989). Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclast-like cells *in vitro*. J. Bone Miner. Res. 4, 113.

Picarella, D., Kratz, A., Li, C.-b., Ruddle, N. H., & Flavell, R. A. (1992). Insulitis in transgenic mice expressing TNF-β (lymphotoxin) in the pancreas. Proc. Natl. Acad. Sci. USA 89, 10036.

Pillai, S., Bikle, D. D., Eessalu, T. E., Aggarwal, B. B., & Elias, P. M. (1989). Binding and biological effects of tumor necrosis factor a on cultured human neonatal foreskin keratinocytes. J. Clin. Invest. 83(3), 816-821.

Pober, J. S., LaPierre, L. A., Stolpen, A. H., Brock, T. A., Springer, T. A., Fiers, W., Bevilacqua, M. P., Mendrick, D. L., & Gimbrone, M. A., Jr. (1987). Activation of cultured human endothelial cells by recombinant lymphotoxin: Comparison with tumor necrosis factor and interleukin 1 species. J. Immunol. 138(10), 3319-3324.

Pociot, F., Molvig, J., Wagensen, L., Worsaal, H., Dalboye, H., Baek, L., & Nerup, L. (1991). Tumor necrosis factor-β gene polymorphism in relation to monokine secretion and insulin dependent diabetes mellitus. Scand. J. Immunol. 33, 37-49.

Powell, M. B., Mitchell, D., Lederman, J., Buckmeier, J., Zamvill, S. S., Graham, M., Ruddle, N. H., & Steinman, L. (1990). Lymphotoxin and tumor necrosis factor-α production by myelin basic protein-specific T cell clones correlates with encaphalitogenicity. Int. Immunol. 2, 539-544.

Powrie, F. & Coffman, R. L. (1993). Cytokine regulation of T-cell function: Potential for therapeutic intervention. Immunol. Today 14(6), 270-274.

Pujol-Borrell, R., Todd, I., Doshi, M., Bottazzo, G. F., Sutton, R., Gray, D., Adolf, G. R., & Feldmann, M. (1987). HLA class II induction in human islet cells by inteferon-gamma plus tumor necrosis factor on lymphotoxin. Nature 326, 304-306.

Ransom, J. H., Evans, C. H., McCabe, R. P., Pomato, N., Heinbaugh, J. A., Chin, M., & Hanna, M. G. (1985). Leukoregulin, a direct-acting anti-cancer immunological hormone that is distinct from lymphotoxin and interferon. Cancer Res. 45, 851-862.

Richter, J. (1990). Lymphotoxin induces secretion of granule proteins from adherent neutrophils: Possible role of intracellular free calcium. J. Leukoc. Biol. 47, 506-513.

Rieckmann, P., Albrecht, M., Kitze, B., Weber, T., Tumani, H., Broocks, A., Luer, W., & Poser, S. (1994). Cytokine mRNA levels in mononuclear blood cells from patients with multiple sclerosis. Neurology 44(8), 1523-1526.

Rockett, K. A., Awburn, M. M., Aggarwal, B. B., Cow, W. B., & Clark, I. A. (1992). In vivo induction of nitrite and nitrate by TNF, LT and IL-1: Possible roles in malaria. Infect. Immun. 60(9), 3725-3730.

Rosenau, W. & Moon, H. D. (1961). Lysis of homologous cells by sensitized lymphocytes in tissue culture. J. Natl. Cancer Inst. 27, 471-483.

Rothe, M., Wong, S. C., Henzel, W. J., & Goeddel, D. V. (1994). A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDA tumor necrosis factor receptor. Cell 78, 681-692.

Ruddle, N. H., & Waksman, B. H. (1967). Cytotoxic effect of lymphocyte-antigen interaction in delayed hypersensitivity. Science 157, 1060-1062.

Ruddle, N. H., & Waksman, B. H. (1968). Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. J. Exp. Med. 128, 1267-1269.

Ruddle, N. H., & Schmid, D. S. (1987). The role of lymphotoxin in T-cell mediated cytotoxicity. Ann. Inst. Pasteur. Immunol. 138, 314.

Ruddle, N. H., Bergman, C. M., McGrath, K. M., Lingenheld, E. G., Grunnet, M. L., Padula, S. J., & Clark, R. B. (1990). An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J. Exp. Med. 172, 1193-1200.

Ruddle, N. H., Picarella, D., Kratz, A., Li, C.-b., & Flavell, R. A. (1993). Probing the mechanism of TNF-α(cachectin)- and TNF-β (lymphotoxin)-induced pancreatic inflammation with transgenic mice. Res. Immunol. 144(5), 336-342.

Ruggiero, V., Tavernier, J., Fiers, W., & Baglioni, C. (1986). Induction of the synthesis of tumor necrosis factor receptors by interferon-γ. J. Immunol. 136, 2445.

Sastry, K. J., Raghav, H. R., Pandita, R., Totpal, K., & Aggarwal, B. B. (1990). HIV-1 tat gene induces tumor necrosis factor-β (lymphotoxin) in a human B-lymphoblastoid cell line. J. Biol. Chem. 265(33), 20091-20093.

Sawada, J., Shioiro-Nakano, K., & Osawa, T. (1975). Purification and characterization of guinea pig lymphotoxin produced by lymphnode cells stimulated by phytohaemagglutinin. Transplantation 19, 335.

Saxne, T., Palladino, M. A., Jr., Heinegård, D., Talal, N., & Wollheim, F. A. (1988). Detection of tumor necrosis factor a but not tumor necrosis factor β in rheumatoid arthritis synovial fluid and serum. Arthritis. Rheum. 31(8), 1041-1045.

Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H., Gatanaga, T., Granger, G. A., Lentz, R., Raab, H., Kohr, W. J., & Goeddel, D. V. (1990). Molecular cloning and expression of a receptor for human tumor necrosis factor. Cell 61(2), 361-370.

Scheurich, P., Thomas, B., Ücer, U., & Pfizenmaier, K. (1987). Immunoregulatory activity of recombinant human tumor necrosis factor: Induction of TNF receptors on human T cells and TNF-a mediated enhacement of T cell responses. J. Immunol. 138, 1786.

Schoenfeld, H. J., Poeschl, B., Frey, J. R., Loetscher, H., Hunziker, W., Lustig, A., & Zulauf, M. (1991). Effecient purification of recombinant human tumor necrosis factor β from *Escherichia coli* yields biologically active protein with a trimeric structure that binds to both tumor necrosis factor receptors. J. Biol. Chem. 266, 3863.

Schuchmann, M., Porter, A., Brakebusch, C., Wallach, D., Riethmüller, G., & Engelmann, H. (1994). The p55 TNF receptor discriminates between TNF-α and TNF-β (lymphotoxin). Eur. Cutokine Netw. 5(2), 95.

Sedgwick, J., MacPhee, I. A. M., & Puklavec, M. (1989). Isolation of encephalitogenic $CD4^+$ T cell clones in the rat. Cloning methodology and interferon γ secretion. J. Immunol. Methods 121, 185-196.

Selmaj, K. R., C.S., Cannella, B., & Brosnan, C. F. (1991a). Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. J. Clin. Invest. 87, 949-954.

Selmaj, K., Raine, C. F., Farooq, M., Norton, W. T., & Brosnan, C. F. (1991b). Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. J. Immunol. 147, 1522-1529.

Seow, H.-F., Goh, C. R., Krishnan, L., & Porter, A. G. (1989). Bacterial expression, facile purification and properties of recombinant human lymphotoxin (tumor necrosis factor beta). Biotechnology (N Y) 7, 363-368.

Seregina, T. M., Mekshenkov, M. I., Turetskaya, R. L., & Nedospasov, S. A. (1989). An autocrine growth factor constitutively produced by a human lymphoblastoid B-cell line is serologically related to lymphotoxin (TNF-β). Mol. Immunol. 26, 339.

Shakhov, A. N., Kuprash, D. V., Azizov, M. M., Jongeneel, C. V., & Nedospasov, S. A. (1990). Structural analysis of the rabbit TNF locus, containing the genes encoding TNF-beta (lymphotoxin) and TNF-alpha (tumor necrosis factor). Gene 95(2), 215-221.

Shalaby, M. R., Aggarwal, B. B., Rinderknecht, E., Svedersky, L. P., Finkle, B. S., & Palladino, M. A. (1985). Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumor necrosis factors. J. Immunol. 135(3), 2069-2073.

Shalaby, M. R., Palladino, M. A., Hirabayashi, S. E., Eessalu, T. E., Lewis, G. D., Shepard, H. M., & Aggarwal, B. B. (1987). Receptor binding and activation of polymorphonuclear neutrophils by tumor necrosis factor-α. J. Leukoc. Biol. 41, 196-204.

Shimizu, Y., Nakamura, T., Niki, T., Hemmi, H., & Sugamura, K. (1989). Purification and characterization of a T lymphocyte-derived differentiation-inducing factor for human promyelocytic cell line (HL-60) and its relationship to lymphotoxin. Microbiol. Immunol. 33(6), 489-501.

Shinjo, K., Tsuda, S., Hayami, T., Asahi, T., & Kawaharada, H. (1989). Increase in permeability of human endothelial cell monolayer by recombinant human lymphotoxin. Biochem. Biophys. Res. Commun. 162(3), 1431-1437.

Smith, D., Gowen, M., & Mundy, G. R. (1987). Effects of interferon gamma and other cytokines on collagen synthesis in fetal rat bone cultures. Endocrinology 120, 2494.

Smith, D., Gowen, M., & Mundy, G. R. (1987). Effects of interferon gamma and other cytokines on collagen synthesis in fetal rat bone cultures. Endocrinology 120, 2494.

Smith, C. A., Davis, T., Anderson, D., Solam, L., Beckmann, M. P., Jerzy, R., Dower, S. K., Cosman, D., & Goodwin, R. G. (1990). A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 248(4958), 1019-1023.

Smith, C. A., Davis, T., Wignall, J. M., Din, W. S., Farrah, T., Upton, C., McFadden, G., & Goodwin, R. G. (1991). T2 open reading frame from the Shope fibroma virus encodes a soluble form of the TNF receptor. Biochem. Biophys. Res. Commun. 176, 335-342.

Smith, C. A., Gruss, H.-J., Davis, T., Anderson, D., Farrah, T., Baker, E., Sutherland, G. R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., Grabstein, K. H., Gliniak, B., McAlister, I. B., Fanslow, W., Alderson, M., Falk, B., Gimpel, S., Gillis, S., Din, W. S., Goodwin, R. G., & Armitage, R. J. (1993). CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 73, 1349-1360.

Spies, T., Morton, C. C., Nedospasov, S. A., Fiers, W., Pious, D., & Strominger, J. L. (1986). Genes for the tumor necrosis factors α and β are linked to the human major histocompatibility complex. Proc. Natl. Acad. Sci. USA 83, 8699-8702.

Stamenkovic, I., Clark, E. A., & Seed, B. (1989). A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. EMBO J. 8, 1403-1410.

Stauber, G. B., & Aggarwal, B. B. (1989). Characterization and affinity cross linking of receptors for human recombinant lymphotoxin (TNF-β) on a human histiocytic lymphoma cell line U937. J. Biol. Chem. 264(6), 3573-3576.
Steffen, M., Ottmann, O. G., & Moore, M. A. S. (1988). Simultaneous production of tumor necrosis factor-α and lymphotoxin by normal T cells after induction with Il-2 and anti-T3. J. Immunol. 140(8), 2621-2624.
Stemme, S., Fager, G., & Hansson, G. K. (1990). MHC class II antigen expression in human vascular smooth muscle cells is induced by interferon-gamma and modulated by tumour necrosis factor and lymphotoxin. Immunology 69, 243-249.
Stone-Wolff, D. S., Yip, Y. K., Kelker, H., Le, J., DeStefano, D. H., Rubin, B., Rinderknecht, E., Aggarwal, B. B., & Vilcek, J. (1984). Interrelationship of human interferon-γ with lymphotoxin and monocyte cytotoxin. J. Exp. Med. 159, 828-843.
Suda, T., Takahashi, T., Golstein, P., & Nagata, S. (1993). Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 75, 1169-1178.
Sung, S.-S., Bjorndahl, J., Wang, C. Y., Kao, H. T., & Fu, S. M. (1988). Production of tumor necrosis factor/cachectin by human T-cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody. J. Exp. Med. 167, 937.
Sung, S.-S. J., Jung, L. K. L., Walters, J. A., Jeffes, E. W. B., Granger, G. A., & Fu, S. M. (1989). Production of lymphotoxin by isolated human tonsillar B lymphocytes and B lymphocyte cell lines. J. Clin. Invest. 84, 236.
Symington, F. W. (1989). Lymphotoxin, tumor necrosis factor and gamma interferon are cytostatic for normal human keratinocytes. J. Invest. Dermatol. 92(6), 799-805.
Takahashi, K., Satoh, J., Seino, H., Zhu, X. P., Sagara, M., Masuda, T., & Toyota, T. (1993). Prevention of type I diabetes with lymphotoxin in BB rats. Clin. Immunol. Immunopathol. 69(3), 318-323.
Takeda, K., Iwamoto, S., & Takeda, M. (1994). Lymphotoxin and TNF differ greatly in capacity to induce differentiation of human myeloblastic leukemia ML-1 cells. Biochem. Mol. Biol. Int. 32(6), 1109-1119.
Tartaglia, L. A., Ayres, T. M., Wong, G. H., & Goeddel, D. V. (1993). A novel domain within the 55 kd TNF receptor signals cell death. Cell 74(5), 845-853.
Tavernier, J., van Ostade, X., Hauquier, G., Prang, T., Lasters, I., de Maeyer, M., Lewit-Bentley, A., & Fourme, R. (1989). Conserved residues of tumour necrosis factor and lymphotoxin constitute the framework of the trimeric structure. FEBS Lett. 257(2), 315-318.
Trinchieri, G., Kobayashi, M., Rosen, M., Loudon, R., Murphy, M., & Perussia, B. (1986). Tumor necrosis factor and lymphotoxin induce differentiation of human myeloid cell lines in synergy with immune interferon. J. Exp. Med. 164, 1206-1255.
Tschachler, E., Robert-Gurroff, M., Gallo, R. C., & Reitz, M. S. (1989). Human T-lymphotropic virus I-infected T cells constitutively express lymphotoxin *in vitro*. Blood 73, 194.

Tsujimoto, M., & Vilcek, J. (1986). Tumor necrosis factor receptors in HeLa cells and their regulation by interferon-γ. J. Biol. Chem. 261, 5384.

Tsujimoto, M., Yip, Y. K., & Vilcek, J. (1986a). Interferon-γ enhances expression of cellular receptors for tumor necrosis factor. J. Immunol. 136, 2441.

Tsujimoto, M., Feinman, R., & Vilcek, J. (1986b). Differential effects of type IFN and IFN-γ on the binding of tumor necrosis factor to receptors in two human cell lines. J. Immunol. 137, 2272.

Turetskaya, R., Fashena, S. J., Paul, N. L., & Ruddle, N. H. (1992). In: *Tumor Necrosis Factor: Structure, Function and Mechanism of Action* (Aggarwal, B. B., & Vilcek, J., Eds.). Marcel Dekker, New York.

Turner, M., & Feldmann, M. (1988). Comparison of patterns of expression of tumor necrosis factor. Lymphotoxin and interleukin-6 mRNA. Biochem. Biophys. Res. Commun. 153(3), 1144-1151.

Udalova, I. A., Nedospasov, S. A., Webb, G. C., Chaplin, D. D., & Turetskaya, R. L. (1993). Highly informative typing of the human TNF locus using six adjacent polymorphic markers. Genomics 16, 180-186.

Ulich, T. R., del Castillo, J., Keys, M., & Granger, G. A. (1987). Recombinant human alpha lymphotoxin (tumor necrosis factor-beta) induces peripheral neutrophilia and lymphopenia in the rat. Am. J. Pathol. 128(1), 5-12.

Upton, C., Macen, J. L., Schreiber, M., & McFadden, G. (1991). Myxoma virus expresses a secreted protein with homology to the tumor necrosis factor receptor gene family that contributes to viral virulence. Virology 184, 370-382.

Van Ostade, X., Tavernier, J., & Fiers, W. (1988). Two conserved tryptophan residues of tumor necrosis factor and lymphotoxin are not involved in the biological activity. FEBS Lett. 238(2), 347-352.

Verjans, G. M. G. M., Van der Linden, S. M., Van Eys, G. J. J. M., de Waal, L. P., & Kijstra, A. (1991). Restriction fragment length polymorphism of the tumor necrosis factor region in pateints with ankylosing spondylitis. Arthritis. Rheum. 34, 486-489.

Voigt, C. G., Maurerfogy, I., & Adolf, G. R. (1992). Natural human tumor necrosis factor β (lymphotoxin) - variable O-glycosylation at Thr7, proteolytic processing, and allelic variation. FEBS Lett. 314(1), 85-88.

Ware, C. F., Crowe, P. D., Grayson, M. H., Androlewicz, M. J., & Browning, J. L. (1992). Expression of surface lymphotoxin and tumor necrosis factor on activated T. B. and natural killer cells. J. Immunol. 149, 3881.

Webb, G. C., & Chaplin, D. D. (1990). Genetic variability at the human tumor necrosis factor loci. J. Immunol. 145, 1278-1285.

Weil, D., Brosset, S., & Dautry, F. (1990). RNA processing is a limiting step for murine tumor necrosis factor β expression in response to interleukin-2. Mol. Cell. Biol. 10(11), 5865-5875.

Williams, T. W. & Bellanti, J. A. (1984). In vitro protective and enhancing effects of interferons from several species on human lymphotoxin-induced target cell killing. Cell Immunol. 83, 255-261.

Williams, T. W., & Granger, G. A. (1968). Lymphocyte *in vitro* cytotoxicity: Lymphotoxins of several mammalian species. Nature 219, 1076-1077.

Williamson, B. D., Carswell, E. A., Rubin, B. Y., Prendergast, J. S., & Old, L. J. (1983). Human tumor necrosis factor produced by human B-cell lines: Synergistic

cytotoxic interaction with human interferon. Proc. Natl. Acad. Sci. USA 80, 5397-5401.

Wong, G. H. W., & Goeddel, D. V. (1986). Tumor necrosis factors α and β inhibit virus replication and synergize with interferons. Nature 323, 819-822.

Xia, H.-Z., Kannapell, C. C., Fu, S. M., & Sung, S-S. J. (1993). Differential regulation of human B-lymphocyte tumor necrosis factor-α (TNF-α) and lymphotoxin (TNF-β) production by protein phosphatase 1 and 2A inhibitor. Blood 82(9), 2806-2812.

Yamashita, K., Ikenaka, Y., Kakutani, T., Kawaharada, H., & Watanabe, K. (1990). Comparison of human lymphotoxin gene expression in CHO cells directed by genomic DNA or cDNA sequences. Agric. Biol. Chem. 54(11), 2801-2809.

Yan, S. D., & Huang, C. C. (1991). Lymphotoxin in human middle ear cholesteatoma. Laryngoscope 101(4 Pt 1), 411-415.

Zhang, L., & Aggarwal, B. B. (1994). Role of sulfhydryl groups in induction of cell surface down-modulation and shedding of extracellular domain of human TNF receptors in human histiocytic lymphoma U937 cells. J. Immunol. 153, 3745-3754.

Zhang, L., Higuchi, M., Totpal, K., Chaturvedi, M. M., & Aggarwal, B. B. (1994). Staurosporine induces the cell surface expression of both forms of human tumor necrosis factor receptors on myeloid and epithelial cells and modulates ligand-induced cellular response. J. Biol. Chem. 269(14), 10270-10279.

Zola, H. (1989). Effect of recombinant human tumour necrosis factor beta (TNFβ) on activation, proliferation and differentiation of human B lymphocytes. Immunology 67, 231-236.

Zucali, J. R., Broxmeyer, H. E., Gross, M. A., & Dinarello, C. A. (1988). Recombinant human tumor necrosis factors α and β stimulate fibroblasts to produce hemopoietic growth factors *in vitro*. J. Immunol. 140(3), 840-844.

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IL6, IL11, LIF, OSM, CARDIOTROPHIN-1, AND CNTF:

AN EXAMPLE OF A CYTOKINE FAMILY SHARING SIGNAL TRANSDUCING RECEPTOR COMPONENTS

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I. INTRODUCTION

Many of the known cytokines and interleukins can be classified as belonging to one of three distinct families. Thus, interleukin-2 (IL2), IL4, IL7, IL9, IL13, and IL15 comprise one family (Lin et al., 1995); IL3, IL5, and granulocyte-macrophage colony stimulatory factor (GMCSF) comprise a second family (Nicola and Metcalf, 1991); IL6, IL11, leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin-1 (CT-1), and ciliary neurotrophic factor (CNTF) comprise a third family (Stahl and Yancopoulos, 1993, 1994). The receptor systems used by all of these cytokines utilize multi-subunit structures. The most important feature which links members of a given cytokine family involves the sharing of receptor components: all cytokines within a given family share at least one of their receptor components. The receptors and signaling pathways utilized by the IL6/IL11/LIF/OSM/CT-1/CNTF family of cytokines are in some respects the best understood, and are reviewed here as an example of the multi-subunit receptor systems used by different members of a cytokine family. Many aspects of receptor function and signal activation for this cytokine family are clearly relevant for other cytokines.

In the following review we will briefly discuss the overlaps and differences in the biological specificities and actions of the different members of the IL6/IL11/LIF/OSM/CT-1/CNTF family. We will then detail the specific and shared receptor subunits used by these cytokines which clearly explain the specificity of action of individual members of the family. We will also explain how these ligands trigger assembly of their multi-subunit receptor complexes, and how this assembly results in activation of intracellular signaling. The first intracellular signaling event involves activation of one or more of the Jak/Tyk tyrosine kinases, which are also utilized by most other cytokines; the Jak kinases appear

to be constitutively associated with the intracellular domains of receptor components in an inactive state, and become activated upon dimerization of the receptor components. We will review recent data that reveals how different families of cytokines, using the same Jak kinases, can elicit very different responses by activating distinct sets of intracellular signaling substrates; it seems as if this specificity results because the receptor components themselves can modulate the selection of substrates by the rather generic Jak kinases. Finally, we will discuss some of the intracellular substrates which are activated. While these include the high-profile members of the STAT family of transcription factors, we will point out that other substrates are as critical for eliciting the cytokine response.

II. RECOGNITION OF THE GP130 CYTOKINE FAMILY

Early work on IL6, which was mostly contributed by Kishimoto and his colleagues (1992), provided a paradigm for subsequent investigations. They found that IL6 could bind to a cell surface receptor called IL6Rα (Yamasaki et al., 1988). But the cytoplasmic domain of IL6Rα was very small, and formation of this complex did not initiate signal transduction. Subsequently, they found that the complex of IL6 bound to IL6Rα could interact with a 130 kDa glycoprotein that they called gp130, which contained a single transmembrane domain and appeared to function as a signal transducer for IL6 (Taga et al., 1989). Thus, IL6 could not bind gp130 directly, and first had to interact with the IL6Rα before engaging gp130 to initiate signaling. Although gp130 had a substantial cytoplasmic domain of 350 amino acids, it lacked obvious signaling motifs such as protein kinase domains, and neither the mechanism of receptor activation nor the mode of signal transduction was obvious.

The realization that CNTF, LIF, OSM, and IL6 formed a distinct cytokine family came from several separate discoveries. Comparison of the amino acid sequence of these cytokines indicated that they were only distantly related, sharing from 6-18% identity and 20-30% similarity (Bazan, 1991). However, LIF, OSM, CNTF, IL6, and another cytokine called granulocyte colony stimulating factor (GCSF) were predicted to have a similar secondary and tertiary structure that conformed to the general pattern of 4 helical bundle cytokines exemplified by growth hormone (Bazan, 1991; Rose and Bruce, 1991). The crystal structures of LIF (Robinson et al., 1994) and CNTF (McDonald et al., 1995) have since been solved; this analysis indeed confirms that these two proteins

share both the general features predicted for the cytokine family, as well as other particular structural characteristics that distinguish them from the other cytokine families.

Simultaneous to the structural prediction, cloning (Davis et al., 1991) of a CNTF-binding protein (CNTFRα) unexpectedly revealed that its closest relative was IL6Rα , while cloning of a low affinity binding protein for LIF (called LIFR) revealed that its closest relative was the IL6 signal transducer, gp130 (Gearing et al., 1991). More in depth investigation of the receptor systems for CNTF, LIF, and OSM led to the realization that they all shared gp130 as a common receptor component, providing the final piece of evidence linking them as members of a single cytokine family (Gearing et al., 1992; Ip et al., 1992). Subsequently, both interleukin 11 (Fourcin et al., 1994; Lu et al., 1994) and cardiotrophin-1 (Pennica et al., 1995a, 1995b) were shown to utilize gp130 as a signal transducing receptor component as well. Although many of the cytokines in this family also require LIFR or a third gp130-related signal transducer known as OSMR (see following), both of which function as equal signal transducing partners with gp130 to initiate signaling upon receptor activation as outlined below (Davis et al., 1993; Stahl et al., 1994; Stahl and Yancopoulos, 1993), we will refer to these cytokines collectively as the gp130 cytokine family since that is the primary common feature by which they are united. Interestingly, the amino acid sequence of IL6 is most closely related to that for granulocyte colony stimulating factor (GCSF), and one might argue that GCSF should be included in this cytokine family as well (Souza et al., 1986). But GCSF does not utilize gp130, instead requiring a gp130-related receptor called GCSFR (Fukunaga et al., 1990; Larsen et al., 1990). It remains a possibility that other undiscovered members of this cytokine family may exist which, in an analogous fashion to the known members as outlined below, utilize both gp130 and GCSFR as receptor components. In addition, other members of this cytokine family may yet be found that use gp130-related signal transducers in the absence of gp130 itself.

III. BIOLOGICAL SPECIFICITIES AND ACTIONS OF THE DIFFERENT MEMBERS OF THE GP130 CYTOKINE FAMILY

Members of the gp130 cytokine family exhibit a wide array of overlapping and distinct actions throughout the body. LIF has extraordinarily widespread sites of biological action, and gives rise to diverse cellular

responses this was reflected in the many different names given to the molecule before it was cloned and characterized (Gough and Williams, 1989; Hilton and Gough, 1991; Kurzrock et al., 1991; Metcalf, 1992; Ryffel, 1993). Some of these activities included induction of M1 myeloid cell differentiation, induction of cholinergic neuronal differentiation, inhibition of ES cell differentiation, stimulation of hepatic acute phase protein synthesis, stimulation of bone formation, promotion of survival of primitive hematopoietic precursors, and inhibition of lipoprotein lipase activity.

The activities of LIF overlap with those of IL6, IL11, and CNTF. IL6 is active on a variety of hematopoietic cells including M1 cells, and also causes stimulation of platelet production, proliferation of myeloma and plasmacytoma cells, stimulation of T cells and bone marrow progenitor cells, and induction of the acute phase response (Kishimoto, et al., 1992). IL11 also causes stimulation of proliferation of certain plasmacytomas and myeloma cells, potentiation of megakaryocytes in combination with other cytokines, inhibition of adipogenesis, and stimulation of osteoclastogenesis (Yang, 1993). In contrast, CNTF is most active only on cells of the nervous system, promoting survival of chick ciliary neurons, induction of the cholinergic switch in sympathetic neurons, and survival of embryonic motor neurons, sensory neurons, and hippocampal neurons (Ip and Yancopoulos, 1992; Stahl and Yancopoulos, 1994). CNTF also acts on the glial cells of the nervous system (Rudge et al., 1994), promoting the *in vitro* differentiation of glial progenitors into astrocytes (Hughes et al., 1988), as well as the maturation and survival of cultured oligodendrocytes. CNTF provided *in vivo* is able to prevent the degeneration of retinal photoreceptor cells (LaVail et al., 1992), as well as the loss of axotomized motor neurons in the facial nucleus of newborn rats, and it has positive effects in mice suffering from a heritable form of progressive motor neuron disease (Sendtner et al., 1992). CNTF is equivalent to LIF in its ability to maintain the pluripotentiality of cultured embryonic stem cells (Conover et al., 1993), and it has myotrophic actions on skeletal muscle *in vivo* (Helgren et al., 1994). Furthermore, mice injected with a cell line that overexpresses a secreted version of CNTF causes cachexia in a similar fashion to both LIF and IL6 (Henderson et al., 1994).

Despite the panoply of actions of these cytokines, knockout mice that are deficient for many of these cytokines show surprisingly normal phenotypes. LIF deficient mice are viable, although females are sterile due to a defect in embryo implantation (Stewart et al., 1992), and the

animals also display decreased numbers of hematopoietic stem cells (Escary et al., 1993). In contrast, injection of LIF or cells that secrete LIF into animals results in cachectic weight loss and death within weeks (Metcalf and Gearing, 1989; Waring et al., 1995). IL6-deficient mice are resistant to ovarectomy-induced osteoporosis (Poli et al., 1994), providing strong support for the proposed role of increased levels of IL6 upon osteoclastogenesis in post-menopausal women (Horowitz, 1993; Jilka et al., 1992; Manolagas and Jilka, 1995). IL6-deficient mice are also resistant to the acute inflammatory response observed upon injection of turpentine, but develop a relatively normal inflammatory response upon injection of lipopolysaccharide (Fattori et al., 1994). The IL6 knockout mice are also somewhat more sensitive to infection by *Listeria* and *vaccinia* virus, consistent with the immunostimulatory role of IL6 (Kopf et al., 1994). Knock-out mice deficient in CNTF display only a limited phenotype that consists of a small loss in motor neurons in aged animals (Dechiara et al., 1995; Masu et al., 1993). Remarkably, a recent study has revealed that about 2-3% of the Japanese population is homozygous for null alleles of CNTF, without exhibiting any notable neurologic abnormalities (Takahashi et al., 1994). However, the generation of mice lacking CNTFRα die at birth and exhibit multiple neurologic defects, consistent with the notion that there is yet another CNTF-like factor to be discovered that also acts via CNTFRα, and that this factor is much more critical than CNTF (Dechiara, et al., 1995). Similarly, mice lacking either gp130 (Yoshida et al., 1993) or LIFR (Ware et al., 1995) die during development or shortly after birth and exhibit multiple abnormalities; the fact that the more severe phenotypes resulting from lack of receptors as compared to individual factors is consistent with the findings that the receptors are shared by multiple ligands, and that the ligands may in some cases have overlapping, redundant, or compensatory actions (Dechiara, et al., 1995).

IV. THE GP130 CYTOKINE FAMILY USES MULTICOMPONENT RECEPTORS

Figure 1 shows a model detailing the cytokine-receptor complexes for every member of the gp130 cytokine family. The elucidation of these receptor models provided a basis for the specific but overlapping sites of action of these cytokines, as well as the similarity in the types of signal transduction pathways that are activated (Stahl and Yancopoulos, 1994).

Receptor Complexes for the gp130 Cytokine Family

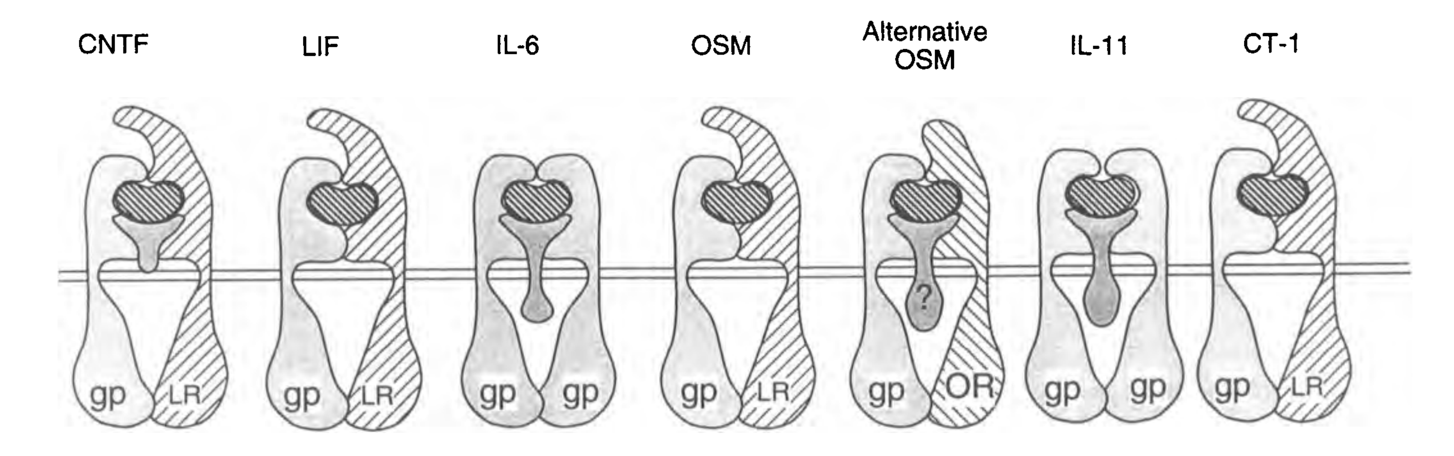


Figure 1. Receptor complexes for the gp130 cytokine family. The composition of the α and signal transducing receptor components are indicated for each member of the cytokine family, where gp indicates gp130, LR indicates LIFR. The alternative OSM signal transducing receptor component is indicated by OR; it is not yet clear whether this receptor complex requires an α component, although there is no evidence that one exists. Although the IL11 complex has not been demonstrated to use two molecules of gp130, it is shown that way by analogy to the other members of the cytokine family.

Moreover, these models provided a rationale for the mechanism by which receptor activation occurs for the gp130 cytokine family, and perhaps for all cytokines in general, as will be detailed in the next section.

In general terms, all of these cytokine receptor complexes contain two signal transducing receptor components, and some of the cytokines also require a specificity determining "α" component (Stahl and Yancopoulos, 1993). Thus, the cytokine-receptor complexes contain either homodimers of gp130 (Davis, et al., 1993; Murakami et al., 1993), or heterodimers of gp130 with another gp130-related signal transducer (Davis et al., 1993). LIF requires LIFR and gp130 as signal transducing receptor components (Baumann et al., 1993; Davis et al., 1993). CNTF also requires LIFR and gp130 as signal transducers, but first must bind to CNTFRα, which functions as a specificity determining receptor component (Davis et al., 1993). The finding that LIF and CNTF both utilized two signal transducing components led to the proposal that IL6 would similarly require two molecules of gp130 (Ip et al., 1992), which was borne out by subsequent investigations (Davis et al., 1993; Murakami et al., 1993). IL11 likewise requires a recently defined specificity-determining α component (Hilton et al., 1994) in addition to gp130 (Fourcin et al., 1994; Lu et al., 1994). Although it has not been rigorously proven, it is likely that IL11 also induces homodimerization of gp130 by analogy to IL6 and the other members of the cytokine family. There are two different functional receptor complexes for OSM: one is identical to the LIF receptor and contains gp130 and LIFR (Baumann et al., 1993; Gearing et al., 1992; Liu, 1992), while the other contains a distinct LIFR-related signal transducer (OSMR) in addition to gp130 (Thoma et al., 1994).

Like, OSM, CT-1 also apparently uses the identical receptor components as LIF (Pennica et al., 1995a, 1995b). While LIF has a signal sequence and is secreted through the traditional ER/Golgi pathway (Gough et al., 1988; Yamamori et al., 1989), CT-1 has no signal sequence (Pennica et al., 1995a)—it may correspond to a non-secreted analog of LIF that is released by an alternative pathway or through loss of cellular integrity. Likewise, CNTF does not have a signal sequence and accumulates in the cytoplasm of certain cells; the mechanism by which it is released is not known. Interestingly, recent comparison of the phenotype of knockout animals for CNTF and CNTFR suggests the existence of another cytokine that uses CNTFR (see previously); by analogy to LIF and CT-1 one might anticipate that it would be secreted.

A common feature of this cytokine family is that each member binds to only one particular receptor component with low affinity in the

absence of the other receptor subunits, but binds with high affinity in the presence of all the required receptor components. CNTF, IL6, and IL11 bind to their specificity-determining α components with nanomolar affinity (Davis et al., 1991; Hilton et al., 1994; Yamasaki et al., 1988), but do not detectibly bind to the signal transducers gp130 or LIFR in the absence of their cognate α components (Davis et al., 1993; Stahl et al., 1993; Taga et al., 1989). Both LIF and CT-1 bind to LIFR in the absence of gp130 (Gearing et al., 1991; Pennica et al., 1995b), while OSM binds weakly to gp130 in the absence of LIFR (Gearing et al., 1992).

The elucidation of these receptor models provided a basis to explain the cell-specific action of each member of the cytokine family. The expression of gp130 is ubiquitous, being found on nearly every cell type that has been examined (Ip et al., 1993; Taga et al., 1989). LIFR expression is also widespread (Ip et al., 1993), although there are cells that express gp130 that do not express LIFR. Thus, LIF is a widely acting cytokine with pleiotrophic effects on numerous tissues throughout the body as described above. But CNTFRα expression is restricted; it is found in cells of the nervous system and in only a few tissues in the periphery, most notably muscle (Davis et al., 1991; Ip et al., 1993). Thus, in a sense, CNTF is a cell-specific version of LIF; only those cells that express CNTFRα in addition to functional LIF receptors (consisting of LIFR and gp130) will be responsive to CNTF (Ip et al., 1993). These findings explain why every cell that is CNTF responsive is also LIF-responsive. Likewise, only those cells that express IL6Rα or IL11Rα in addition to the ubiquitously expressed gp130 will be responsive to IL6 or IL11 respectively. Thus, the α components function as specificity-determining receptor components that allow a cell to selectively respond to a given cytokine, which then activates a common signal transduction pathway through activation of gp130, or gp130 and LIFR.

V. LIGAND-MEDIATED RECEPTOR ASSEMBLY

A major question that arises from the receptor models in Figure 1 is whether the cytokines bind to a preassembled multicomponent receptor complex, or whether cytokine binding initiates assembly of the receptor complex. This was investigated through reconstruction experiments in COS cells in which epitope-tagged receptor components were expressed alone or in combination, and assessed for their ability to co-immunoprecipitate in the absence or presence of the cytokine (Davis et al., 1993).

None of the receptor components co-immunoprecipitated in a complex in the absence of ligand. However, addition of the cytokine-induced assembly of a receptor complex, so that immunoprecipitation of gp130 resulted in co-precipitation of the other receptor component(s) (Davis et al., 1993). Thus, CNTF could induce formation of a complex between gp130 and LIFR only in the presence of its specificity-determining component CNTFRα. Surprisingly, CNTF also induced formation of a stable complex between CNTFRα and gp130 even in the absence of LIFR (Davis et al., 1993). However, this complex did not give activation and subsequent tyrosine phosphorylation of the gp130, and represents a stable intermediate for receptor complex formation, but includes only one signal transducing component and is therefore not signal transduction competent.

These experiments suggested a general model for cytokine receptors (Stahl and Yancopoulos, 1993) in which the receptor components are initially unassociated and complex formation is driven by cytokine binding (Figure 2). Cytokines such as CNTF and IL6 first bind to their required α specificity-determining component, and this complex then recruits one signal transducing receptor component to form an unactivated intermediate; recruitment of the second signal transducing component then initiates receptor activation and subsequent signal transduction events. Alternatively, it is possible that two of these intermediates, each containing one molecule of cytokine, α component and signal transducing component, dimerize to form the activated receptor complex. Recently, evidence in favor of the latter model has been presented for IL6 (Paonessa et al., 1995; Ward et al., 1994). Regardless of which model is correct, it is evident that dimerization of the signal transducing components, a common feature of both models, gives rise to receptor activation. Cytokines such as LIF which do not require an α component bind first to one of the signal transducing components before recruiting the second signal transducing component to initiate signal transduction (Gearing et al., 1991, 1992). Thus, cytokine receptors are analogous to many receptor tyrosine kinases, where ligand binding induces dimerization of the receptor to initiate signal transduction (Schlessinger and Ullrich, 1992).

How does cytokine binding drive formation of the receptor complex? One possibility is that complex formation between the cytokine and the α component induces conformational changes in either the cytokine or the receptor or both that favors subsequent interaction with the signal transducing components. A second possibility is that there are relatively

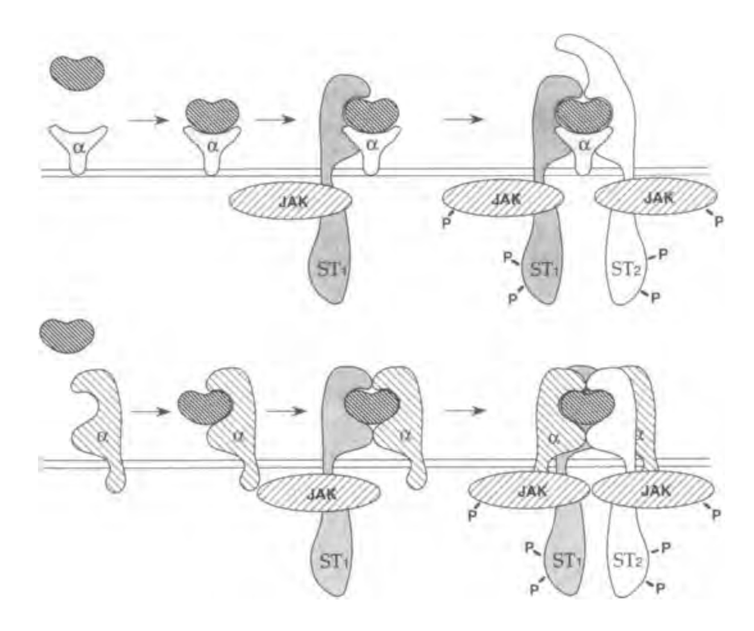


Figure 2. Two models for the activation of CNTF, IL6, and IL11 receptor complexes. The cytokine binds to its receptor components in an ordered manner: first binding to the α component before recruiting in a stepwise fashion each of the signal transducing (ST) receptor components. The ST components are preassociated with an inactive form of members of the JAK/TYK family members of tyrosine kinases. Dimerization of the ST components allows transphosphorylation and activation of the JAK kinase, with subsequent tyrosine phosphorylation (P) of the ST components. The bottom panel shows an alternative model (as described in the text) in which the final activated receptor complex is hexameric, containing two molecules each of the cytokine, α receptor component and signal transducers, which is formed through dimerization of the cytokine I•α•ST intermediate. In either case, homo- or heterodimerization of the JAK kinase bound to the ST components initiates signal tranduction.

weak individual interactions between both the cytokine and the signal transducers, as well as the α component and the signal transducers. Although these individual interactions are too weak to drive binding between the individual receptor components, or between the cytokine and the signal transducers in the absence of the α component, the complex of the cytokine with its α would present a combined molecular surface that constituted a high affinity binding site for the signal transducers.

Crystallographic studies of growth hormone (GH) bound to its receptor are consistent with the latter mechanism, in which each molecule of GH has two receptor binding sites, one of which is high affinity while the other is lower affinity (de Vos et al., 1992). However, the GH receptors also have a low affinity binding site for each other, so that binding of GH to the first receptor molecule forms a combined surface that forms a high affinity binding site for a second receptor component (de Vos et al., 1992). There is no evidence for substantial conformational changes in either the cytokine or the receptor upon binding, although some plasticity in the conformation of the growth hormone bound to the prolactin receptor has been observed (Kossiakoff et al., 1994). Although fewer details are known for the gp130 cytokine family, the existing evidence is consistent with the GH model. Cross-linking experiments in COS cells showed that CNTF could be cross-linked to gp130 and LIFR only in the presence of CNTFRα, suggesting that the cytokine interacts directly with the signal transducers in the receptor complex (Stahl et al., 1993). Furthermore, mutational analysis of IL6 reveals that patches of amino acids form sites that interact with either IL6Rα or gp130 (Brakenhoff et al., 1994; Paonessa et al., 1995; Savino et al., 1994). In addition, some IL6 mutants will bind to IL6Rα but fail to drive interaction with gp130 and thus function as antagonists (Brakenhoff et al., 1994; Savino et al., 1994). Mutants of IL6Rα also exist that bind normally to IL6, but fail to interact with gp130 and thus also function as antagonists (Savino et al., 1993; Yawata et al., 1993). Taken together, these data suggest that, like the GH system, both cytokine and the a component possess relatively independent weak binding sites for the signal transducing components that together form a high-affinity binding surface following initial complex formation.

An amazing consequence of these models is the promiscuity shown by gp130, and to a lesser extent LIFR. According to this model, gp130 must be able to interact with six different cytokines, three distinct α components, and at least two other signal transducing receptor compo-

nents in addition to homodimerizing with itself. Sequence alignment of the cytokines do not reveal any obvious conserved residues that could constitute a "gp130 binding site." Nor are the residues of IL6 that are implicated in interacting with gp130 conserved among the other members of the gp130 cytokine family. This may suggest that there is a certain level of plasticity in the gp130 binding site, and that there are a variety of different amino acid motifs that, when presented on the cytokine, can serve as a relatively low affinity site for binding to gp130. One might also suspect that, given the demands of interacting with so many ligands, gp130 would be highly conserved across species. In fact, gp130 is only 77% conserved between humans and rodents.

VI. RECEPTOR COMPLEX ASSEMBLY LEADS TO ACTIVATION OF SIGNALING INVOLVING RECEPTOR-ASSOCIATED JAKS

Although gp130 and LIFR do not possess cytoplasmic sequences corresponding to protein kinase domains, early studies revealed that inhibitors of protein kinases could block cytokine-induced gene inductions (Campbell et al., 1993; Ip et al., 1992). Furthermore, subsequent experiments showed that addition of IL6, CNTF, or LIF to responsive cells resulted in rapid tyrosine phosphorylation of LIFR and/or gp130 (Ip et al., 1992; Murakami et al., 1991; Stahl et al., 1993), as well as other unidentified intracellular proteins. It was thus hypothesized that the signal transducing receptor components were non-covalently associated with non-receptor tyrosine kinases. Furthermore, the finding that receptor activation resulted from homo- or heterodimerization of the signal transducing components (Davis et al., 1993; Murakami et al., 1993) fit nicely with this idea, since by analogy to receptor tyrosine kinases, dimerization of tyrosine kinases bound to the signal transducing components could allow for their activation.

The discovery that the Jak/Tyk family of tyrosine kinases are involved in cytokine signaling provided a major advance to the field (Argetsinger et al., 1993; Silvennoinen et al., 1993b; Velazquez et al., 1992; Witthuhn et al., 1993). Members of this unusual family of non-receptor tyrosine kinases were initially cloned in homology-based strategies intended to identify new tyrosine kinases (Firmbach-Kraft et al., 1990; Kawamura et al., 1994; Wilks, 1989; Wilks et al., 1991). The four known members of this family—Jak1, Jak2, Jak3, and Tyk2—all share the distinctive

feature of having a second kinase-like domain of unknown function in addition to the more canonical tyrosine kinase domain positioned at their carboxy terminus. Elegant studies involving complementation of genetic defects in cells that had been selected for loss of responsiveness to the interferons (IFN) revealed that Jak1 and Tyk2 were required for IFN-α signaling (Muller et al., 1993; Velazquez et al., 1992), while Jak1 and Jak2 were required for functional responses to IFN-γ (Muller et al., 1993; Watling et al., 1993). The possibility that the Jak/Tyk kinases were generally involved in responses to many different cytokines came with the observation that Jak2 could be activated in response to both erythropoietin and growth hormone (Argetsinger et al., 1993; Witthuhn et al., 1993).

Subsequent studies revealed that the gp130 cytokine family also utilized the Jak/Tyk kinases, and provided a paradigm for the mechanism of their activation (Lutticken et al., 1994; Stahl et al., 1994). These experiments showed that the Jak/Tyk kinases could pre-associate with the signal transducing receptor components gp130 or LIFR in the absence of the cytokine (Stahl et al., 1994), but became tyrosine phosphorylated and activated only after addition of the cytokine induced homo- or heterodimerization of the signal transducing receptor components to which they were bound (Figure 3). Preassociation of the Jak/Tyk kinases was found to occur at the membrane proximal regions of gp130 and LIFR (Stahl et al., 1994); sequences in this region had previously been shown to be required for gp130 function (Murakami et al., 1991). Moreover, many different cytokine receptors contain homologous proline rich sequences in their membrane proximal regions, suggesting that ligand-mediated dimerziation between signal transducing receptor components pre-associated with Jak/Tyk kinases, as seen with the gp130 cytokine family, might represent a universal mechanism by which all cytokine receptors initiate signaling. This idea has largely proven to be true, although in some cases preassociation of the Jak/Tyk kinases to the receptor may be weak and difficult to detect in the absence of the ligand (Colamonici et al., 1994; Miyazaki et al., 1994; Nicholson et al., 1994; Rui et al., 1994; Russell et al., 1994).

Although there were many similarities between the roles and mechanisms of activation of the Jak/Tyk kinases in other cytokine receptor systems as compared to those used by the gp130 family of cytokines, there were also striking differences (Stahl et al., 1994). While all other cytokines seemed to use only a single Jak/Tyk kinase or only particular combinations of these kinases, members of the gp130 cytokine family

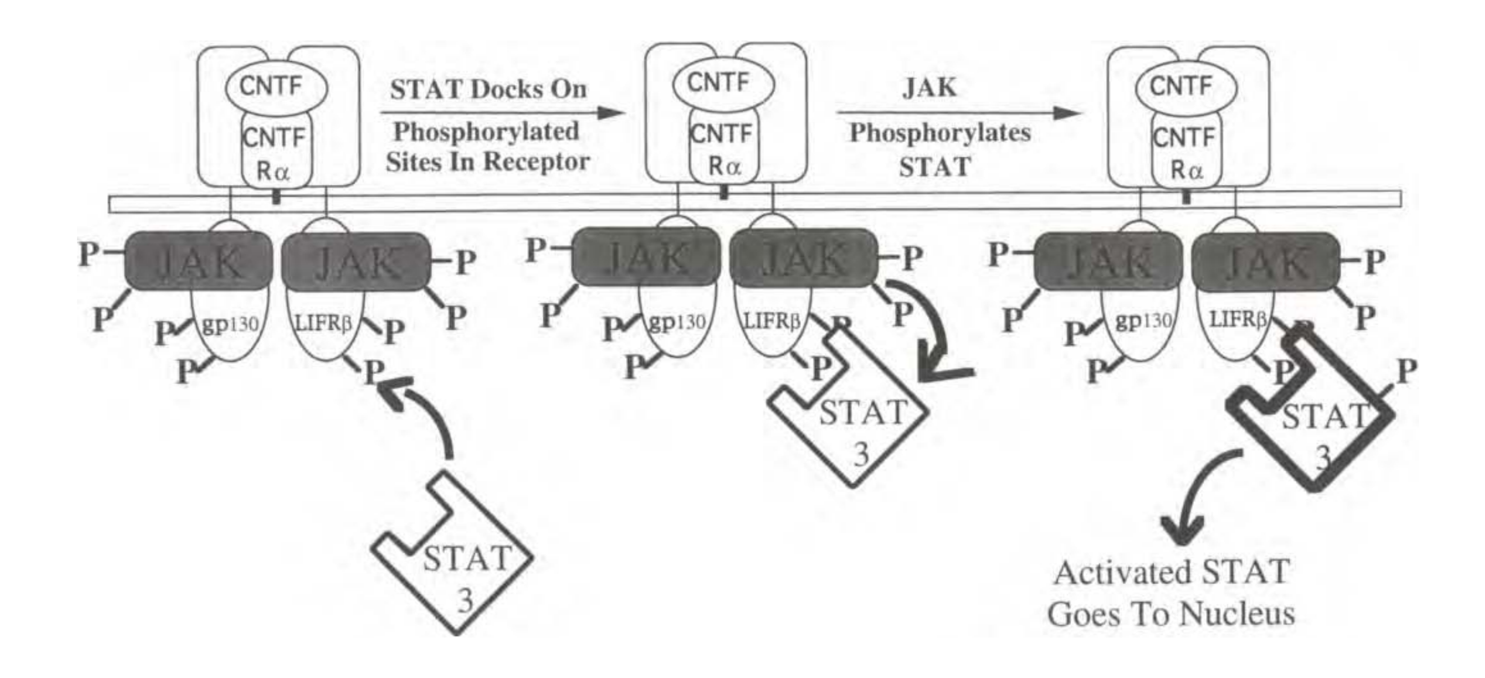


Figure 3. Signal transducing receptor components specify activation of particular pathways. The signal transducers gp130 and LIFR possess tyrosine-based motifs that become tyrosine phosphorylated and bind the SH2 domains of downstream targets such as Stat3. Stat3 binds to these motifs, and becomes tyrosine phosphorylated through action of the associated JAK kinase. The activated Stat3 then dissociates, dimerizes, and moves to the nucleus to activate transcription.

were capable of utilizing all of the Jak/Tyk kinases that were known at the time (Stahl et al., 1994). However, they used different combinations of the Jak/Tyk kinases in different cells—thus, CNTF, LIF, IL6, and OSM similarly activated both Jak1 and Jak2 but not Tyk2 in the EW-1 neuroendocrine cell line, while these cytokines utilized only Jak2 and Tyk2 in a second cell line, or Jak1 and Tyk2 in a third cell line (Stahl et al., 1994). This differential selectivity occurred despite the fact that the expression level of each Jak/Tyk kinase was similar in each of the cell lines. The determinants that govern which Jak/Tyk kinases are activated by the gp130 cytokines in a particular cell line remain unclear. However, it is evident that in a given cell line, all members of this cytokine family use the same combination of the Jak/Tyk kinases. Furthermore, reconstitution experiments in which individual Jak/Tyk kinases were expressed in combination with the signal transducing receptors, revealed that any of the Jak/Tyk kinases, when provided in excess, can allow for functional responses (Stahl et al., 1994). One possible explanation that could account for this variable selectivity of the Jak/Tyk kinases by gp130 and LIFR is that the many different cytokine receptor signal transducing receptor components expressed in a given cell all compete for relatively limiting amounts of the Jak/Tyk kinases, and that thus the particular members of the kinase family pre-associated with gp130 and LIFR will vary from cell to cell depending on what other cytokine receptors are expressed in each cell.

VII. THE GP130 FAMILY OF CYTOKINES ACTIVATE A COMMON SET OF INTRACELLULAR SUBSTRATES

Given the sharing of signal transducing receptor components by the gp130 family of cytokines, one might anticipate that each member of this cytokine family would activate similar intracellular signaling pathways. Examination of the proteins that become inducibly tyrosine phosphorylated in a given cell line in response to CNTF, LIF, OSM, and IL6 revealed that each cytokine induced an identical pattern of phosphorylated products (Boulton et al., 1994; Ip et al., 1992). Likewise, IL11 also induces similar phosphorylation patterns as IL6, OSM, and LIF (Berger et al., 1994). Moreover, this pattern was largely independent of cell type: neurons, fibroblasts, hematopoietic cells, and astrocytes all displayed very similar phosphorylated proteins (Boulton et al., 1994; Ip et al., 1992; Rudge et al., 1994). Thus, despite the fact that IL6 and IL11 homodimer-

izes gp130 while CNTF and LIF heterodimerize gp130 and LIFR, the only difference that was observed was tyrosine phosphorylation of LIFR itself, which is not induced by IL6 or IL11. However, it is possible that further analysis may reveal that the cytokines utilizing LIFR may activate unique pathways.

Perhaps the most widely investigated signal transduction pathway that is activated by the gp130 cytokine family involves the activation of several members of the family of proteins known as STATs: signal transducers and activators of transcription (Darnell et al., 1994). STATs exist in the cytoplasm in an inactive form (Schindler et al., 1992), but then become tyrosine phosphorylated upon receptor activation and subsequently form homo- or hetero-dimers (Shuai et al., 1994), translocate to the nucleus and bind directly to particular DNA sequences to stimulate transcription of responsive genes (Darnell et al., 1994). The gp130 family of cytokines preferentially activate Stat3 (Akira et al., 1994; Boulton et al., 1995b; Lutticken, 1994; Zhong et al., 1994), but also activate Stat1 in some, but not all, cell types (Akira et al., 1994; Bonni et al., 1993; Boulton et al., 1995b; Feldman et al., 1994; Symes et al., 1994). Stat3 activation accounts for the induction of many of the acute phase genes in hepatic cells, thus explaining its pseudonym: acute phase response factor (Akira et al., 1994; Kopf et al., 1994; Wegenka et al., 1993). Although some receptor tyrosine kinases such as that for epidermal growth factor are reported to weakly activate Stat3 (Zhong et al., 1994), and cytokines such as GCSF and IFN-α also activate Stat3 (Boulton, Zhong et al., 1995b; Tian et al., 1994), many other cytokines such as IFN-γ (Boulton et al., 1995b; Zhong et al., 1994) and erythropoietin (Stahl et al., 1995)do not activate Stat3, and instead preferentially activate other members of the STAT family. The molecular basis for this specificity, as well as the role that Stat3 activation might play in different cell types, is described in later sections.

Although Stat3 is a major and important downstream target of the gp130 cytokine family, activation of Stat3 does not explain all of the cellular consequences following cytokine administration. For example, proliferation of BAF cells can be driven with mutated versions of gp130 (Murakami et al., 1991; Narazaki et al., 1994) that retain the ability to activate the Jak kinases, but are no longer capable of giving Stat3 activation (see following). Thus, activation of additional cellular pathways can have profound effects.

Analysis of other signal transduction pathways activated by the gp130 family of cytokines revealed both similarities and differences to those

pathways known to be activated by other cytokine families or receptor tyrosine kinases (Boulton et al., 1994). Thus, the gp130 cytokine family induces tyrosine phosphorylation of targets such as phospholipase Cγ, the pp120 src substrate, the p110 phosphatidylinositol 3-kinase component, protein tyrosine phosphatase 1D (PTP1D), SHC, and the extracellular signal-regulated kinases (also known as map kinases) ERK1 and ERK2 (Boulton et al., 1994). Although phospholipase Cγ and PTP1D are also activated by receptor tyrosine kinases, they have not been reported to become activated in response to other cytokines.

The differences between the signaling pathways activated by the gp130 cytokine family compared to factors that activate receptor tyrosine kinases probably account for the existence of synergistic effects that are observed on some cells upon stimulation with combinations of these factors. This synergy between different classes of factors can have very important biological consequences. CNTF or LIF together with fibroblast growth factor have been shown to act collaboratively on sympathoadrenal progenitor cells to promote their terminal differentiation to NGF-dependent post-mitotic neurons (Ip et al., 1994). LIF has also been shown to display similar collaborative abilities with NGF during the differentiation of neural crest precursors into sensory neurons (Murphy et al., 1993); LIF or CNTF can also synergize with BDNF to promote survival of motor neurons *in vitro* (Wong et al., 1993). Similar dramatic interactions are observed during hematopoiesis: the IL6 pathway can synergize with the lignad for the c-Kit receptor tyrosine kinase (*steel* or stem cell factor) to efficiently drive expansion of CD34+ progenitor cells (Sui et al., 1995). Furthermore, therapeutic implications of such synergistic interactions have already been explored *in vivo*. For example, survival of a mouse suffering from a genetic motor neuron disease is synergistically enhanced by a combination of CNTF and brain-derived neurotrophic factor (BDNF) versus treatment with either factor alone (Mitsumoto et al., 1994).

The synergy described previously upon phenotypic cellular responses to combinations of members of the gp130 cytokine family with other factors is also observed at the molecular level for the activation of the ERKs (Boulton et al., 1995a, Ip et al., 1994). The ERKs have proven to be an important kinase family that is widely activated by many types of factors and hormones, resulting in many changes in cellular function including induction of gene transcription (Boulton et al., 1991). In many cells, ERK activation by RTK factors such as FGF, NGF, and insulin occurs very rapidly (2-3 mins) after addition of the factor and subsides

relatively quickly. In contrast, CNTF, LIF, and IL6 stimulation of the same cell results in a much slower timeframe of ERK activation, peaking at 10-15 mins, and also subsides more slowly. Addition of both the gp130 cytokine and the RTK factor simultaneously results in a more profound and prolonged level of ERK activation than that observed with either factor alone (Boulton et al., 1995a; Ip et al., 1994). Interestingly, it has been demonstrated that ERK activation by the gp130 cytokines occurs by a distinct pathway compared to the RTK factors; ERK activation by CNTF or LIF can be inhibited by the serine/threonine kinase inhibitor H7, which has no effect on ERK activation by FGF, NGF, or insulin (Boulton et al., 1994). Although the exact pathway by which ERK is activated by the gp130 cytokines is not yet resolved, this difference may account for the molecular synergy that is observed with RTK factors on ERK activation, and may contribute to the observed phenotypic synergy as well.

VIII. WITH ALL THE CONVERGENCE ON JAKS BY DIFFERENT CYTOKINE FAMILIES, HOW DO YOU GET SIGNALING SPECIFICITY?

The results described previously revealed that the members of the gp130 cytokine family all activate different JAKs in different cells, yet inevitably give rise to activation of the same signal transduction pathways despite this promiscuity. Furthermore, a variety of different cytokines activate the same set of Jaks as does the gp130 cytokine family, but stimulate a distinct set of intracellular targets. For example, IFN-γ activates Stat1 and IFN-α activates Stat1, Stat2, and Stat3 (Boulton et al., 1995b; Darnell et al., 1994), whereas the gp130 cytokine family all preferentially activate Stat3 (Akira et al., 1994; Boulton et al., 1995b; Wegenka et al., 1993; Zhong et al., 1994). One possibility was that the Jaks might determine the choice of downstream substrates: definition of the particular Jaks and Stats differentially activated by the interferons led to proposals that Jak1 specifically activates Stat1, whereas Tyk2 specifically targets STAT2 (Ihle et al., 1994; Silvennoinen et al., 1993a). However, the gp130 cytokine family consistently induces phosphorylation of Stat3 despite activating different JAKs in different cells (Boulton et al., 1994, 1995b; Stahl et al., 1994). Thus, it was proposed that the cytokine receptor signal transducing components not only contain a cytoplasmic JAK-binding domain, but also selectively bind distinct

substrates allowing them to be activated by the associated JAK (Greenlund et al., 1994; Stahl et al., 1994; Stahl and Yancopoulos, 1993). This proposal was also consistent with findings that a tyrosine-containing motif in the IFNγ receptor was required for Stat1 activation by this receptor, and that phosphorylated peptides containing this motif could directly bind Stat1 (Greenlund et al., 1994).

Experiments with the gp130 cytokine family revealed that, consistent with the second proposal described above (Figure 3), tyrosine-based motifs in the cytoplasmic domains of the signal transducing components gp130 and LIFR are the critical determinants that direct activation of particular signaling targets (Stahl et al., 1995). These experiments used chimeric receptors consisting of extracellular domains from receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) or TrkC that were fused to the intracellular domains of gp130 or LIFR. Thus, dimerization and activation of the intracellular domains could be driven by addition of EGF or neurotrophin 3, (NT3) respectively, facilitating mutational analysis of the gp130 and LIFR cytoplasmic domains even in cells that express wild-type gp130 and LIFR. Examination of chimeric receptors with progressively larger carboxy terminal deletions of the gp130 cytoplasmic domain that successively removed the five distal tyrosines revealed that the ability of the receptor to activate both Stat3 and PTP1D required the presence of particular tyrosine-based motifs in the cytoplasmic domain (Stahl et al., 1995). Thus, deletion of all five of the tyrosines resulted in a receptor which could still preassociate with and activate a JAK kinase, but could not activate Stat3 or PTP1D. Interestingly, addition of a single tyrosine-based motif from gp130 with the sequence YXXQ (where X indicates any amino acid) could restore the receptors' ability to phosphorylate Stat3, but not STAT1 or PTP1D. The presence of a single tyrosine-based motif on the receptors' cytoplasmic domain with the sequence YSTV was sufficient to mediate activation of PTP1D, but not Stat3. Most convincingly, the addition of five amino acids with the sequence GYMPQ (consistent with the YXXQ consensus for Stat3 specification) to the carboxy-terminal end of the receptor for erythropoietin, which by itself does not normally give rise to activation of Stat3, now endowed that receptor with the ability to mediate Stat3 phosphorylation (Stahl et al., 1995). Taken together, these results demonstrate that short tyrosine-containing motifs within the cytoplasmic domains of cytokine receptors can determine the substrates activated by the JAKs, which appears to act in a rather generic fashion and thus depend on receptor-dictated specifications. Further-

more, these short tyrosine-containing motifs appear relatively modular, as they can be appended at several positions within the receptor and still specify substrate choice. Current data suggest that these motifs become phosphorylated on tyrosine by the JAK, and then act to recruit substrates to the receptor complex via the binding of SH2 domains within these substrates to the phosphorylated motifs (Figure 3; Heim et al., 1995; Stahl et al., 1995).

IX. WHAT ACCOUNTS FOR DISTINCT RESPONSES BY DIFFERENT CELL TYPES TO A SINGLE CYTOKINE?

While the mechanisms that govern the signaling specificity of different cytokine receptors are beginning to emerge as described previously, other questions of specificity remain to be elucidated. For example, Stat1 and Stat3 can both bind to the same DNA sequences *in vitro* and *in vivo* (Gronowski et al., 1995; Zhong et al., 1994), yet cytokines which activate these STATs do not always give rise to induction of the same genes. Recent data suggest that Stat3 and Stat1 have subtle differences in their DNA-binding specificities, allowing them to work on different target genes (Horvath et al., 1995; Seidel et al., 1995). In addition, the observation that the STATs can heterodimerize (Shuai et al., 1994) as well as interact with other accessory proteins (Pellegrini and Schindler, 1993) may create distinct target gene specificities for different STATs. Interestingly, recent data also indicates that while tyrosine phosphorylation of the STATs is sufficient for their translocation to the nucleus and binding to DNA, serine phosphorylation of STATs is required for maximal activation of some gene responses (Boulton et al., 1995b; Wen et al., 1995). Thus, this serine phosphate may modulate interactions with accessory proteins.

Even more puzzling is the fact that CNTF, LIF, and IL-6 share signal transducers and the Jak/Tyk kinases as well as many of their downstream signal mediators such as Stat3 and PTP1D, yet each cytokine is capable of eliciting such strikingly different types of responses in different cells. That is, while CNTF and LIF have apparently identical actions on shared neuronal targets, as would be expected based on their receptor structures, how can LIF elicit such dramatically different responses from other cell types—such as proliferation in hemopoietic precursors, acute phase responses from the liver, bone deposition from osteoblast cells, and myeloid differentiation in myeloid progenitor cells? Furthermore, every

type of neuron responds differently to CNTF itself—a sympathetic neuron undergoes a cholinergic switch in response to CNTF, whereas a ciliary neuron does not. The only conclusion that can be reached is that each cell, and each different class of neuron, must be programmed to respond differently to the same signal—but where does differential interpretation of the cell surface signal begin? It could certainly begin near the receptors themselves—different cells may have different intracellular substrates available to be activated by the receptor-associated tyrosine kinase. But this is certainly not the whole story—as mentioned previously, members of the CNTF cytokine family seem to activate many of the same substrates, such as Stat3 and Stat1, in all cells examined—whether it be neuronal or non-neuronal. So how can some of these universally activated substrates, such as Stat3 and Stat1, be activated in both a liver cell and a neuron, but ultimately lead to completely different gene responses (i.e., acute phase response gene responses vs. neuronal differentiation marker gene responses) and phenotypic effects in these two cells? This question is particularly confusing in light of the observation that the acute phase response genes activated in the liver cell and neuronal marker genes activated in the neuron both have binding sites for the STATs. One likely possibility is that the different sets of response genes are in two different states of readiness in the two cell types—as a result of distinct differentiation programs, only the acute phase response genes are "accessible" or "competent" to be activated by the STATs in the liver cell, while the neuronal differentiation genes are somehow "hidden" or "inaccessible" in these cells; the opposite would be true in the neuron, and furthermore different classes of neurons would have different sets of response genes that were competent to respond. The precise molecular determinants of "accessibility" remain to be determined and could involve cell- and sequence-specific DNA binding proteins, methylation state, and chromatin condensation. In any case, these determinants are presumably an inextricable part of the differentiation process that leads to different cell types as well as to countless different classes of neurons, each capable of responding quite differently even to the same external signal.

X. CONCLUSIONS AND SPECULATIONS

IL-6, IL-11, LIF, OSM, CT-1, and CNTF provide an illustrative example of a cytokine family which is grouped together based on the sharing of

common receptor subunits by all the members. Each of these cytokines triggers assembly of their multi-subunit receptor complexes from components that are initially found unassociated on the cell surface. The dimerization of signal transducing receptor components, which occurs during ligand-induced receptor assembly, appears to be the critical step for the initiation of intracellular signaling. This critical step for signal initiation involves activation of one or more of the JAK/Tyk tyrosine kinases, which are pre-associated with the signal transducing receptor components, but only become activated upon ligand-induced dimerization of these components. The JAK/Tyk kinases seem to be rather generic tyrosine kinases, and the particular substrates they activate appear, in large part, to be determined by the receptor components themselves. This specification is accomplished by modular tyrosine-containing motifs in the receptors, which apparently become phosphorylated by the JAK/Tyk kinases and then in turn serve to recruit substrates to the receptor complex by acting as binding sites for the SH2-domains of these substrates once recruited to the receptor complex, the substrates are acted upon by the JAK/Tyk kinases. While the STATs are the most intensively studied substrates activated by these cytokines, many other substrates are also activated, and these other substrates in fact seem more critical than the STATs for mediating some of the more prominent responses—such as proliferation—induced by these cytokines in some cells. Remarkably, members of the IL-6 family seem to activate similar initial substrates in all the various cells they act upon—the dramatic differences in the response elicited by CNTF from a neuron as compared to the response elicited by IL-6 from a hemopoietic precursor appears to result not from the activation of different initial substrates, but from the manner in which these substrate activations are interpreted within the context of the particular responding cell.

While the details of the receptor systems, JAKs employed, and substrates specified clearly differ for other cytokine families, the same major principles seem to apply. Thus, other cytokines most likely also induce stepwise formation of their receptor complexes, with dimerization (either homo- or hetero-) of JAK-associated receptor components probably serving as the critical initiating step for intracellular signaling. While pre-association of JAKs has only been noted for a small number of other cytokine receptors to date, it should be noted that even in the case of gp130 and LIFR, detection of such JAK pre-associations required development of special cell lysis conditions that did not disrupt the weak pre-associations. The mechanism by which dimerization of cytokine

receptor components activates the associated JAKs remains unknown, but may be similar to the mechanism by which receptors with intrinsic tyrosine kinase activity can be activated following ligand-induced dimerization. As with gp130 and its close relatives, it appears that tyrosine-containing motifs within the receptor components for other cytokine families are critical for specifying substrates to be acted upon by the JAKs. As for the substrates activated by members of the IL-6 family, the cell context in which other cytokines activate their substrates most likely determines how these activations are eventually interpreted, and in the phenotypic responses ultimately elicited. The finding that different families of cytokines activate distinct combinations of substrates, and that these profiles also differ as compared to the substrates activated by factors using receptor tyrosine kinases, probably underlies the abilities of different families of cytokines to synergize with each other, as well as with factors using receptor tyrosine kinases.

While many of the general principles that govern cytokine-mediated receptor assembly and signal activation appear to be coming into focus, further study is sure to reveal new principles and major surprises. In addition, continued efforts should also serve to illustrate how these processes can either be exploited or manipulated so as to positively intervene in disease processes involving these cytokines and their receptors.

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REFERENCES

Akira, S., Nishio, Y., Inoue, M., Wang, X.-J., Wei, S., Matsusaka, T., Yoshida, K., Sudo, T., Naruto, M., & Kishimoto, T. (1994). Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 77, 63-71.

Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N., & Carter-Su, C. (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell 74, 237-244.

Baumann, H., Zieglr, S. F., Mosley, B., Morella, K. K., Pajovic, S., & Gearing, D. P. (1993). Reconstitution of the response to leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor in hepatoma cells. J. Biol. Chem. 268, 8414-8417.

Bazan, J. F. (1991). Neuropoietic cytokines in the hematopoietic fold. Neuron 7, 197-208.

Berger, L. C., Hawley, T. S., Lust, J. A., Goldman, S. J., & G., H. R. (1994). Tyrosine phosphorylation of JAK-TYK kinases in malignant plasma cell lines growth-stimulated by interleukins 6 and 11. Bioch. Biophys. Res. Comm. 202, 596-605.

Bonni, A., Frank, D. A., Schindler, C., & Greenberg, M. E. (1993). Characterization of a pathway for ciliary neurotrophic factor signaling to the nucleus. Science 262, 1575-1579.

Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H., & Yancopoulos, G. D. (1991). ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65, 663-677.

Boulton, T. G., Stahl, N., & Yancopoulos, G. D. (1994). Ciliary neurotrophic factor/leukemia inhibitory factor/interleukin 6/oncostatin M family of cytokines induces tyrosine phosphorylation of a common set of proteins overlapping those induced by other cytokines and growth factors. J. Biol. Chem. 269, 11648-11655.

Boulton, T. G., Stahl, N., & Yancopoulos, G. D. (1995a). Unpublished data.

Boulton, T. G., Zhong, Z., Wen, Z., Darnell Jr., J. E., Stahl, N., & Yancopoulos, G. D. (1995b). STAT3 activation by cytokines utilizing gp130 and releated transducers involves a secondary modification requiring an H7-sensitive kinase. Proc. Natl. Acad. Sci USA 92, 6915-6919.

Brakenhoff, J. P., de Hon, F. D., Fontaine, V., ten Boekel, E., Schooltink, H., Rose-John, S., Heinrich, P. C., Content, J., & Aarden, L. A. (1994). Development of a human interleukin-6 receptor antagonist. J. Biol. Chem. 269, 86-93.

Campbell, G. S., Christian, L. J., & Carter-Su, C. (1993). Evidence for involvement of the growth hormone receptor-associated tyrosine kinase in actions ofgrowth hormone. J. Biol. Chem. 268, 7427-7434.

Colamonici, O. R., Uyttendaele, H., Domanski, P., Yan, H., & Krolewski, J. J. (1994). $p135^{tyk2}$, and interferon-α-activated tyrosine kinase, is physically associated with and interferon-α receptor. J. Biol. Chem. 269, 3518-3522.

Conover, J. C., Ip, N. Y., Poueymirou, W. T., Bates, B., Goldfarb, M. P., DeChiara, T. M., & Yancopoulos, G. D. (1993). Ciliary neurotrophic factor maintains the pluripotentiality of embryonic stem cells. Development 119, 559-565.

Darnell, J. E., Kerr, I. M., & Stark, G. R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

Davis, S., Aldrich, T. H., Stahl, N., Taga, T., Kishimoto, T., Ip, N. Y., & Yancopoulos, G. D. (1993). LIFRβ and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. Science 260, 1805-1808.

Davis, S., Aldrich, T. H., Valenzuela, D. M., Wong, V., Furth, M. E., Squinto, S. P., & Yancopoulos, G. D. (1991). The receptor for ciliary neurotrophic factor. Science 253, 59-63.

de Vos, A. M., Ultsch, M., & Kossiakoff, A. A. (1992). Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. Science 255, 306-312.

Dechiara, T. M., Vejsada, R., Pouyemirou, W. T., Acheson, A., Suri, C., Conover, J. C., Friedman, B., McClain, J., Pan, L., Stahl, N., Ip, N. Y., Kato, A., & Yancopoulos,

G. D. (1995). Mice lacking the CNTF receptor, unlike mice lacking CNTF, exhibit profound motor neuron deficits at birth. Cell. In press.
Escary, J. L., Perreau, J., Dumenil, D., Ezine, S., & Brulet, P. (1993). Leukemia inhibitory factor is necessary for maintenance of haematopoietic stem cells and thymocyte stimulation. Nature 363, 361-364.
Fattori, E., Cappelletti, M., Costa, P., Sellitto, C., Cantoni, L., Carelli, M., Faggioni, R., Fantuzzi, G., Ghezzi, P., & Poli, V. (1994). Defective inflammatory response in interleukin-6 deficient mice. J. Exp. Med. 180, 1243-1250.
Feldman, G. M., Petricoin III, E. F., David, M., Larner, A. C., & Finbloom, D. S. (1994). Cytokines that associate with the signal trnasducer gp130 activate the interferon-induced transcription factor p91 by tyrosine phosphorylation. J. Biol. Chem. 269, 10747-10752.
Firmbach-Kraft, I., Byers, M., Shows, T., Dalla-Favera, R., & Krolewski, J. J. (1990). tyk2, prototype of a novel class of non-receptor tyrosin kinase genes. Oncogene 5, 1329-1336.
Fourcin, M., Chevalier, S., Lebrun, J. J., Kelly, P., Pouplard, A., Wijdenes, J., & Gascan, H. (1994). Involvement of gp130/interleukin-6 receptor transducing component in interleukin-11 receptor. Eur. J. Immun. 24, 277-280.
Fukunaga, R., Ishizuka-Ikeda, E., Seto, Y., & Nagata, S. (1990). Expression cloning of a receptor for murine granulocyte colony-stimulating factor. Cell 61, 341-350.
Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., McGourty, J., Brasher, K. K., King, J. A., Gillis, S., Mosley, B., Ziegler, S. F., & Cosman, D. (1992). The IL-6 signal transducer, gp130: An oncostatin M receptor and affinity converter for the LIF receptor. Science 255, 1434-1437.
Gearing, D. P., Thut, C. J., VandenBos, T., Gimpel, S. D., Delaney, P. B., King, J., Price, V., Cosman, D., & Beckmann, M. P. (1991). Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130. EMBO J. 10, 2839-2848.
Gough, N. M., & Williams, R. L. (1989). The Pleiotropic Actions of Leukemia Inhibitory Factor. Cancer Cells-A Monthly Review 1, 77-80.
Gough, N. M., Gearing, D. P., King, J. A., Willson, T. A., Hilton, D. J., Nicola, N. A., & Metcalf, D. (1988). Molecular cloning and expression of the human hologue of the murine gene encoding meyeloid leukemia-inhibitory factor. Proc. Natl. Acad. Sci. USA 85, 2623-2627.
Greenlund, A. C., Farrar, M. A., Viviano, B. L., & Schreiber, R. D. (1994). Ligand induced IFNγ receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). EMBO 13, 1591-1600.
Gronowski, A. M., Zhong, Z., Wen, Z., Thomas, M. J., Darnell, J. E. J., & Rotwein, P. (1995). *In vivo* growth hormone treatment rapidly stimulates the tyrosine phosphorylation and activation of Stat3. Mol. Endocrin. 9, 171-177.
Heim, M. K., Kerr, I. M., Stark, G. R., & Darnell, J. E. (1995). Contribution of STAT SH2 groups to specific interferon signaling by the Jak-STAT pathway. Science 267, 1347-1349.
Helgren, M. E., Squinto, S. P., Davis, H. L., Parry, D. J., Boulton, T. G., Heck, C. S., Zhu, Y., Yancopoulos, G. D., Lindsay, R. M., & DiStefano, P. S. (1994). Trophic effects of ciliary neurotrophic factor on denervated skeletal muscle. Cell 76, 493-504.

Henderson, J. T., Seniuk, N. A., Richardson, P. M., Gauldie, J., & C., R. J. (1994). Systemic administration of ciliary neurotrophic factor induces cachexia in rodents. J. Clin. Invest. 93, 2632-2638.

Hilton, D. J., & Gough, N. M. (1991). Leukemia Inhibitory Factor: A biological perspective. J. Cell. Biochem. 46, 21-26.

Hilton, D. J., Hilton, A. A., Raicevic, A., Rakar, S., Harrison-Smith, M., Gough, N. M., Begley, C. G., Metcalf, D., Nicola, N. A., & Willson, T. A. (1994). Cloning of a murine IL-11 receptor α-chain; requirement for gp130 for high affinity binding and signal transduction. EMBO J. 13, 4765-4775.

Horowitz, M. C. (1993). Cytokines and estrogen in bone: Anti-osteoporotic effects. Science 260, 626-627.

Horvath, C. M., Wen, Z., & Darnell, J. E. J. (1995). A STAT protein domain that determines DNA sequence recognition suggests a novel DNA-binding domain. Genes & Devel. 9, 984-994.

Hughes, S. M., Lillien, L. E., Raff, M. C., Rohrer, H., & Sendtner, M. (1988). Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture. Nature 335, 70-73.

Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B., & Silvennoinen, O. (1994). Signaling by the cytokine receptor superfamily: JAKs and STATs. TIBS 19, 222-227.

Ip, N. Y., Boulton, T. G., Li, Y., Verdi, J. M., Birren, S. J., Anderson, D. J., & Yancopoulos, G. D. (1994). CNTF, FGF, and NGF collaborate to drive the terminal differentiation of MAH cells into post-mitotic neurons. Neuron 13, 443-455.

Ip, N. Y., McClain, J., Barrezueta, N. X., Aldrich, T. H., Pan, L., Li, Y., Wiegand, S. J., Friedman, B., Davis, S., & Yancopoulos, G. D. (1993). The α chain of the CNTF receptor is required for signalling and defines potential CNTF targets in the adult and during development. Neuron 10, 89-102.

Ip, N. Y., Nye, S. N., Boulton, T. G., Davis, S., Taga, T., Li, Y., Birren, S. J., Yasukawa, K., Kishimoto, T., Anderson, D. J., Stahl, N., & Yancopoulos, G. D. (1992). CNTF and LIF act on neuronal cells via shared signalling pathways that involve the IL-6 signal transducing receptor component gp130. Cell 69, 1121-1132.

Ip, N. Y., & Yancopoulos, G. D. (1992). Ciliary Neurotrophic Factor and its receptor complex. Progress in Growth Factor Research 4, 139-155.

Jilka, R. L., Hangoc, G., Girasole, G., Passeri, G., Williams, D. C., Abrams, J. S., Boyce, B., Broxmeyer, H., & Manolagas, S. C. (1992). Increased osteoclast development after estrogen loss: Mediation by interleukin-6. Science 257, 88-91.

Kawamura, M., McVicar, D. W., Johnston, J. A., Blake, T. B., Chen, Y.-Q., Lal, B. K., Lloyd, A. R., Kelvin, D. J., Staples, J. E., Ortaldo, J. R., & O'Shea, J. J. (1994). Molecular cloning of L-Jak, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. Proc. Natl. Acad. Sci USA 91, 6374-6378.

Kishimoto, T., Akira, S., & Taga, T. (1992). Interleukin-6 and its receptor: A paradigm for cytokines. Science 258, 593-597.

Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H., & Kohler, G. (1994). Impaired immune and acute-phase responses in interleukin-6 deficient mice. Nature 368, 339-342.

Kossiakoff, A. A., Somers, W., Ultsch, M., Andow, K., Muller, Y. A., & De Vos, A. M. (1994). Comparison of the intermediate complexes of human growth hormone bound to the human growth hormone and prolactin receptors. Protein Science 3, 1697-1705.

Kurzrock, R., Estrov, Z., Wetzler, M., Gutterman, J. U., & Talpaz, M. (1991). LIF: Not just a leukemia inhibitory factor. Endocrine Reviews 12, 208-217.

Larsen, A., Davis, T., Curtis, B. M., Gimpel, S., Sims, J. E., Cosman, D., Park, L., Sorensen, E., March, C. J., & Smith, C. A. (1990). Expression cloning of a human granulocyte colony-stimulating factor receptor—A structural mosaic of hematopoietin receptor, immunoglobulin, and fibronectin domains. J. Exp. Med. 172, 1559-1570.

LaVail, M. M., Unoki, K., Yasumura, D., Matthes, M. T., Yancopoulos, G. D., & Steinberg, R. H. (1992). Multiple growth factors, cytokines and neurotrophins rescue photoreceptors from the damaging effects of constant light. Proc. Natl. Acad. Sci USA 89, 11249-11253.

Lin, J.-X., Migone, T.-S., Tsang, M., Friedmann, M., Weatherbee, J. A., Zhou, L., Yamauchi, A., Bloom, E. T., Mietz, J., John, S., & Leonard, W. J. (1995). The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity 2, 331-339.

Liu, J., Modrell, B., Aruffo, A., Marken, J. S., Taga, T., Yasukawa, K., Murakami, M., Kishimoto, T., Shoyab, M. (1992). Interleukin-6 signal transducer gp130 mediates oncostatin M signaling. J. Biol. Chem. 267, 16763-16766.

Lu, Z. Y., Zhang, X. G., Gu, Z. J., Yasukawa, K., Amiot, M., Etrillard, M., Bataille, R., & Klein, B. (1994). A highly sensitive quantitative bioassay for human interleukin-11. J. Immun. Meth. 173, 19-26.

Lutticken, C., Wegenka, U. M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A. G., Wilks, A. F., Yasukawa, K., Taga, T., Kishimoto, T., Barbieri, G., Pellegrini, S., Sendtner, M., Heinrich, P. C., & Horn, F. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science 263, 89-92.

Manolagas, S. C., & Jilka, R. L. (1995). Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N. Eng. J. Med. 332, 305-311.

Masu, Y., Wolf, E., Holtmann, B., Sendtner, M., Brem, G., & Thoenen, H. (1993). Disruption of the CNTF gene results in motor neuron degeneration. Nature 365, 27-32.

McDonald, N. Q., Panayotatos, N., & Hendrickson, W. A. (1995). Crystal structure of dimeric human ciliary neurotrophic factor determined by MAD phasing. EMBO J. 14, 2689-2699.

Metcalf, D. (1992). Leukemia inhibitory factor—a puzzling polyfunctional regulator. Growth Factors 7, 169-173.

Metcalf, D., & Gearing, D. P. (1989). Fatal syndrome in mice engrafted with cells producing high levels of the leukemia inhibitory factor. Proc. Natl. Acad. Sci. USA 86, 5948-5952.

Mitsumoto, H., Ikeda, K., Klinkosz, B., Cedarbaum, J. M., Wong, V., & Lindsay, R. M. (1994). Arrest of motor neuron disease in *wobbler* mice cotreated with CNTF and BDNF. Science 265, 1107-1110.

Miyazaki, T., Kawahara, A., Fujii, H., Nakagawa, Y., Minami, Y., Liu, Z.-J., Oishi, I., Silvennoinen, O., Witthuhn, B. A., Ihle, J. N., & Taniguchi, T. (1994). Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. Science 266, 1045-1047.

Muller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A. G., Barbieri, G., Witthuhn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ihle, J. N., Stark, G. R., & Kerr, I. M. (1993). The protein tyrosine kinase JAK1 complements defects in interferon-α/β and -γ signal transduction. Nature 366, 129-135.

Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T., & Kishimoto, T. (1991). Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. Proc. Natl. Acad. Sci. USA 88, 11349-11353.

Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T., & Kishimoto, T. (1993). IL6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. Science 260, 1808-1810.

Murphy, M., Reid, K., Hilton, D. J., & Bartlett, P. F. (1993). Involvement of leukemia inhibitory factor and nerve growth factor in the development of dorsal root ganglion neurons. Development 117, 1173-1182.

Narazaki, M., Witthuhn, B. A., Yoshida, K., Silvennoinen, O., Yasukawa, K., Ihle, J. N., Kishimoto, T., & Taga, T. (1994). Activation of JAK2 kinase mediated by the interleukin 6 signal transducer gp130. Proc. Natl. Acad. Sci. USA 91, 2285-2289.

Nicholson, S. E., Oates, A. C., Harpur, A. G., Ziemiecki, A., Wilks, A. F., & Layton, J. E. (1994). Tyrosine kinase JAK1 is associated with the granulocyte-colony-stimulating factor receptor and both become tyrosine-phospohrylated after receptor activation. Proc. Natl. Acad. Sci. 91, 2958-2988.

Nicola, N. A., & Metcalf, D. (1991). Subunit promiscuity among hemopoietic growth factor receptors. Cell 67, 1-4.

Paonessa, G., Graziani, R., De Serio, A., Savino, R., Ciapponi, L., Lahm, A., Salvati, A. L., Toniatti, C., & Ciliberto, G. (1995). Two distinct and independent sites on IL-6 trigger gp130 dimer formation and signalling. EMBO J. 14, 1942-1951.

Pellegrini, S., & Schindler, C. (1993). Early events in signaling by interferons. Trends Biochem. Sci. 18, 338-342.

Pennica, D., King, K. L., Shaw, K. J., Luis, E., Rullamas, J., Luoh, S. M., Darbonne, W. C., Knutzon, D. S., Yen, R., Chien, K. R., Baker, J. B., & Wood, W. I. (1995a). Expression cloning of cardiotrophin 1, a cytokine that induces cardiac myocyte hypertrophy. Proc. Natl. Acad. Sci. USA 92, 1142-1146.

Pennica, D., Shaw, K. J., Swanson, T. A., Moore, M. W., Shelton, D. L., Zioncheck, K. A., Rosenthal, A., Taga, T., Paoni, N. F., & Wood, W. I. (1995b). Cardiotrophin-1. Biological activities and binding to the leukemia inhibitory factor receptor/gp130 signaling complex. J. Biol. Chem. 270, 10915-10922.

Poli, V., Balena, R., Fattori, E., Markatos, A., Yamamoto, M., Tanaka, H., Ciliberto, G., Rodan, G. A., & Costantini, F. (1994). Interleukin-6 defiecient mice are protected from bone loss caused by estrogen depletion. EMBO J. 13, 1189-96.

Robinson, R. C., Grey, L. M., Staunton, D., Vankelecom, H., Vernallis, A. B., Moreau, J.-F., Sturat, D. I., Heath, J. K., & Jones, E. Y. (1994). The crystal structure and

biological funciton of leukemia inhibitory factor: Implications for receptor binding. Cell 77, 1101-1116.

Rose, T. M., & Bruce, G. (1991). Oncostatin M is a member of a cytokine family that includes leukemia-inhibitory factor, granulocyte colony-stimulating factor, and interleukin 6. Proc. Natl. Acad. Sci. USA. 88, 8641-8645.

Rudge, J. S., Li, Y., Pasnikowski, E. M., Mattsson, K., Pan, L., Yancopoulos, G. D., Weigand, S. J., Lindsay, R. M., & Ip, N. Y. (1994). Neurotrophic factor receptors and their signal transduction capabilities in rat astrocytes. Eur. J. Neurosci. 6, 693-705.

Rui, H., Kirken, R. A., & Farrar, W. L. (1994). Activation of receptor-associated tyrosine kinase Jak2 by prolactin. J. Biol. Chem. 269, 5364-5368.

Russell, S. M., Johnston, J. A., Noguchi, M., Kawamura, M., Bacon, C. M., Friedmann, M., Berg, M., McVicar, D. W., Witthuhn, B. A., Silvennoinen, O., Goldman, A. S., Schmalstieg, F. C., Ihle, J. N., O'Shea, J. J., & Leonard, W. J. (1994). Interaction of IL-2Rβ and γc chains with Jak1 and Jak3: Implications for XSCID and XCID. Science 266, 1042-1044.

Ryffel, B. (1993). Pathology induced by leukemia inhibitory factor. Int. Rev. Exp. Path. 34 Pt. B., 69-72.

Savino, R., A., L., Giorgio, M., Cabibbo, A., Tramontano, A., & Ciliberto, G. (1993). Saturation mutagenesis of the human interleukin 6 receptor-binding site: Implications for its three-dimensional structure. Proc. Natl. Acad. Sci. USA 90, 4067-4071.

Savino, R., Lahm, A., Salvati, A. L., Ciapponi, L., Sporeno, E., Altamura, S., Paonessa, G., Toniatti, C., & Ciliberto, G. (1994). Generation of interleukin-6 receptor antagonists by molecular-modeling guided mutagenesis of residues important for gp130 activation. EMBO J. 13, 1357-1367.

Schindler, C., Shuai, K., Prezioso, V. R., & Darnell, J. E., Jr. (1992). Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 257, 809-813.

Schlessinger, J., & Ullrich, A. (1992). Growth factor signaling by receptor tyrosine kinases. Neuron 9, 383-391.

Seidel, H. M., Milocco, L. H., Lamb, P., Darnell, E. J., Jr., Stein, R. B., & Rosen, J. (1995). Spacing of palindromic half sites as a determinant of selective STAT (signal transducers and activators of transcription) DNA binding and transcriptional activity. Proc. Natl. Acad. Sci. USA 92, 3041-3045.

Sendtner, M., Schmalbruch, H., Stockli, K. A., Kreutzberg, G. W., & Thoenen, H. (1992). Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. Nature 358, 502-504.

Shuai, K., Horvath, C. M., Huang, L. H. T., Qureshi, S. A., Cowburn, D., & Darnell, J. E., Jr.(1994). Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. Cell 76, 821-828.

Silvennoinen, O., Ihle, J. N., Schlessinger, J., & Levy, D. E. (1993a). Interferon-induced nuclear signaling by Jak protein tyrosine kinases. Nature 366, 583-585.

Silvennoinen, O., Witthuhn, B., Quelle, F. W., Cleveland, J. L., Yi, T., & Ihle, J. N. (1993b). Structure of the murine JAK2 protein tyrosine kinase and its role in interleukin 3 signal transduction. Proc. Natl. Acad. Sci. USA 90, 8429-8433.

Souza, L. M., Boone, T. C., Gabrilove, J., Lai, P. H., Zsebo, K. M., Murdock, D. C., Chazin, V. R., Bruszewski, J., Lu, H., Chen, K. K., Barendt, J., Platzer, E., Moore, M. A. S., Mertelsmann, R., & Welte, K. (1986). Recombinant human granulocyte colony-stimulating factor: Effects on normal and leukemic myeloid cells. Science 232, 61-65.

Stahl, N., & Yancopoulos, G. D. (1993). The alphas, betas and kinases of cytokine receptor complexes. Cell 74, 587-590.

Stahl, N., & Yancopoulos, G. D. (1994). The tripartite CNTF receptor complex: Activation and signaling involves components shared with other cytokines. J. Neurobiology 25, 1454-1466.

Stahl, N., Davis, S., V., W., Taga, T., Kishimoto, T., Ip, N. Y., & Yancopoulos, G. D. (1993). Crosslinking of CNTF and LIF to endogenous and reconstructed receptor complexes identifies LIF binding protein as a CNTF receptor component. J. Biol. Chem. 268, 7628-7631.

Stahl, N., Boulton, T. G., Farruggella, T., Ip, N. Y., Davis, S., Witthuhn, B., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J. N., & Yancopoulos, G. D. (1994). Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL6 β receptor components. Science 263, 92-95.

Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., & Yancopoulos, G. D. (1995). Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science 267, 1349-1353.

Stewart, C. L., Kaspar, P., Brunet, L. J., Bhatt, H., Gadi, I., Kontgen, F., & Abbondanzo, S. J. (1992). Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. Nature 359, 76-79.

Sui, X., Tsuji, K., Tanaka, R., Tajima, S., Muraoka, K., Ebihara, Y., Idebuchi, K., Yasukawa, K., Taga, T., Kishimoto, T., & Nakahata, T. (1995). gp130 and c-Kit signalings synergize for *ex vivo* expansion of human primitive hemopoietic progenitor cells. Proc. Natl. Acad. Sci USA 92, 2859-2863.

Symes, A., Lewis, S., Corpus, L., Rajan, P., Hyman, S. E., & Fink, J. S. (1994). STAT proteins participate in the regulation of the vasoactive intestinal peptide gene by the ciliary neurotrophic factor family of cytokines. Mol. Endocrin. 8, 1750-1763.

Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirando, T., & Kishimoto, T. (1989). Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 58, 573-581.

Takahashi, R., Yokoji, H., Misawa, H., Hayashi, M., Hu, J., & Deguchi, T. (1994). A null mutation in the human CNTF gene is not causally related to neurological diseases. Nature Genetics 7, 79-84.

Thoma, B., Bird, T. A., Friend, D. J., Gearing, D. P., & Dower, S. K. (1994). Oncostatin M and leukemia inhibitory factor trigger overlapping and different signals through shared receptor complexes. J. Biol. Chem. 269, 6215-6222.

Tian, S. S., Lamb, P., Seidel, H. M., Stein, R. B., & Rosen, J. (1994). Rapid activation of the Stat3 transcription factor by granulocyte colony-stimulating factor. Blood 84, 1760-1764.

Velazquez, L., Fellous, M., Stark, G. R., & Pellegrini, S. (1992). A protein kinase in the interferon a/ß signaling pathway. Cell 70, 313-322.

Ward, L. D., Howlett, G. J., Discolo, G., Yasukawa, K., Hammacher, A., Moritz, R. L., & Simpson, R. J. (1994). High affinity interleukin-6 receptor is a hexameric

complex consisting of two molecules each of interleukin-6, interleukin-6 receptor, and gp130. J. Biol. Chem. 269, 23286-23289.

Ware, C. B., Horowitz, M. C., Renshaw, B. R., Hunt, J. S., Liggit, D., Koblar, S. A., Gliniak, B. C., McKenna, H. J., Papayannopoulou, T., Thoma, B., Cheng, L., Donovan, P. J., Peschon, J. J., Bartlett, P. F., Willis, C. R., Wright, B. D., Carpenter, M. K., Davison, B. L., & Gearing, D. P. (1995). Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. Development. In press.

Waring, P. M., Waring, L. J., Billington, T., & Metcalf, D. (1995). Leukemia inhibitory factor protects against experimental lethal *Escherichia coli* septic shock in mice. Proc. Natl, Acad. Sci USA 92, 1337-1341.

Watling, D., Guschin, D., Muller, M., Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Rogers, N. C., Schindler, C., Stark, G. R., Ihle, J. N., & Kerr, I. M. (1993). Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-γ signal transduction pathway. Nature 366, 166-170.

Wegenka, U. M., Buschmann, J., Lutticken, C., Heinrich, P. C., & Horn, F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. Mol. Cell. Bol. 13, 276-288.

Wen, Z., Zhong, Z., & Darnell, E. J., Jr. (1995). Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell 82, 241-250.

Wilks, A. F. (1989). Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc. Natl. Acad. Sci. USA 86, 1603-1607.

Wilks, A. F., Harpur, A. G., Kurban, R. R., Ralph, S. J., Zurcher, & G., Ziemiecki, A. (1991). Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol. Cell. Biol. 11, 2057-2065.

Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., & Ihle, J. N. (1993). JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 74, 227-236.

Wong, V., Arriaga, R., Ip, N. Y., & Lindsay, R. M. (1993). The neurotrophins BDNF, NT3, NT4/5, but not NGF up-regulate the cholinergic phenotype of developing motor neurons. Eur. J. Neurosci. 5, 466-474.

Yamamori, T., Fukada, K., Aebersold, R., Korsching, S., Fann, M. J., & Patterson, P. H. (1989). The cholinergic neuronal differentiation factor from heart cells is identical to leukemia inhibitory factor. Science 246, 1412-1416.

Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T., & Kishimoto, T. (1988). Cloning and expression of the human interleukin-6 (BSF-2/IFNb 2) receptor. Science 241, 825-828.

Yang, Y-C. (1993). Interleukin 11: An overview. Stem Cells 11, 474-486.

Yawata, H., Yasukawa, K., Natsuka, S., Murakami, M., Yamasaki, K., Hibi, M., Taga, T., & Kishimoto, T. (1993). Structure-function analysis of human IL6 receptor: dissociation of amino acid residues required for IL6-binding and for IL6 signal transduction through gp130. EMBO J. 12, 1705-1712.

Yoshida, K., Saito, M., Suematsu, S., Yoshida, N., Kumanogoh, A., Taga, T., & Kishimoto, T. (1993). Disruption of the gene for shared cytokine signal transducer, gp*130*. Fifth International Conference on Human Leukocyte Differentiation Antigens, 42, p. 339.

Zhong, Z., Wen, Z., & Darnell, J. E. (1994). Stat3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 264, 95-98.

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Edited by **Derek Le Roith,** *Diabetes Branch, NIDDK, National Institutes of Health* and **Carolyn Bondy,** *Developmental Endocrinology Branch, NICHD, National Institutes of Health*

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